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January 11, 2010

Attorney Docket No.: D2033-7060US

Commisioner-for Patents

MAIL STOP: PATENT EXT.

P.O./Box 1450

Alexandria, VA 2231<u>3-1450</u>

Presented for filing is an Application for Extension of Patent Term for:

WILLIAM MARKLAND and ROBERT CHARLES LADNER Inventors:

Patent No.: 5,795,865 B1

Issued: August 18, 1998

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND ANALOGUES THEREOF

Assignee: Dyax Corp.

Enclosed are the following papers, including those required for filing an Application For Extension of Patent Term under 35 U.S.C § 156:

		Pages
Application for	Extension of Patent Term	29
Attachment A	Power of Attorney	7 (including cover page)
Attachment B	Package insert for KALBITOR®	13 (including cover page)
Attachment C	U.S. Patent No.: 7,276,480 B1	35 (including cover page)
Attachment D	Biologics License Approval Letter including enclosures	33 (including cover page)
Attachment E	U.S. Patent No.: 5,795,865	44 (including cover page)
Attachment F	Terminal Disclaimer	3 (including cover page)
Attachment G	Certificate of Correction	5 (including cover page)
Attachment H	Maintenance Fee Status	3 (including cover page)
Attachment I	Alignment of the Kunitz domain of ecallantide and	
	Bovine trypsin protease inhibitor	2 (including cover page) *
Attachment J1	Letter from FDA acknowledging receipt of the first IND	4 (including cover page).
Àttachment J2	Contact report for DYAX-FDA teleconference of	٠
	February 8, 2002	2 (including cover page)
Attachment K	Letter from FDA acknowledging receipt of the second IND	4 (including cover page)



Application for Extension of Patent Term In re US Patent No.: 5,795,865 B1

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Pages

Attachment L Letter from Dyax to the Center for Biologic Evaluation and

Research dated May 31, 2002 which summarized the

May 30, 2002 telephone conference

5 (including cover page)

Attachment M Communication dated June 12, 2008 from Dyax Corp. to the

Center for Drug Evaluation and Research discussing conveyance

of BB-IND#10232 to Cubist Pharmaceuticals

4 (including cover page)

Attachment N Communication from Dyax Corp. to the Center for Drug Evaluation

and Research dated June 13, 2008, in which BB-IND#10426

was amended

8 (including cover page)

Attachment O Letter from FDA acknowledges receipt of the final submission

of the BLA

4 (including cover page)

Attachment P Certification of Copies of Application Papers ( $x^2 - 205$  pgs each)

411 (including cover page)

#### Enclosures:

- -Transmittal letter (original and 1 copy)
- -Postcard listing items (with number of pages for each) in submission
- -check in the amount of \$1,120.00

If there are any questions regarding this filing, it is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at (617) 395-7000.

Kindly acknowledge receipt of this Application for Extension of Patent Term by returning the enclosed postcard.

Please direct all correspondence to the following:

37462 PTO Customer Number

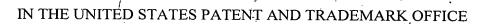
Respectfully submitted,

Laurie Butler Lawrence.

Reg. No. 46,593 Enclosures

LBL/sec

Attorney Docket No.: D2033-7060US/10280-096US1



J.S. Patent No.: 5,795,865

sued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

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CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. §1.10

The undersigned hereby certifies that this document was deposited with the U.S. Postal Service on

January 11, 2010 for express mailing in accordance with §1,6(a)(2).

Laurie Butler Lawrence, Reg. No. 46,593

EB 575684378 US

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Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450 01/13/2010 JADDO1

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### APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Applicant, Dyax Corp., represents that it is the Assignee of the entire interest in and to United States Patent No. 5,795,865 granted to Dyax Corp. on the 18th day of August 1998, for 'Kallikrein-inhibiting "kunitz domain" proteins and analogues thereof' by virtue of an assignment from William Markland and Robert Charles Ladner to Protein Engineering Corporation, recorded in the U.S. Patent and Trademark Office at Reel 008151, Frame 0915, on September 25, 1996, and from Protein Engineering Corporation to Dyax Corp., recorded in the U.S. Patent and Trademark Office at Reel 008535, Frame 0123, on June 4, 1997. The assignment from William Markland and Robert Charles Ladner to Protein Engineering Corporation was re-recorded in the U.S. Patent and Trademark Office at Reel 019353, Frame 0204, on May 30, 2007. The assignment from Protein Engineering Corporation to Dyax Corp. was re-recorded in the U.S. Patent and Trademark Office at Reel 019353, Frame 0229, on May 30, 2007.

By the Power of Attorney enclosed herein (Attachment A), Applicant has

Issued: August 18, 1998

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appointed several individual attorneys, including Laurie Butler Lawrence, as attorneys for Dyax Corp. with regard to this application for extension of the term of U.S. Patent No. 5,795,865 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

Dyax Corp. is the holder of the regulatory approval granted with respect to the regulatory review period relied on herein.

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### Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for extension of the patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. § 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented herein in a format which follows the order of the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

### (1) Identification of the Approved Product [1.740(a)(1)]

The approved product is KALBITOR® (ecallantide). Ecallantide is a recombinant 60 amino acid plasma kallikrein inhibiting protein produced in *Pichia pastoris* cells. KALBITOR® is supplied as a sterile, clear, colorless liquid which is free of preservatives for subcutaneous administration. The approved product is described in more detail in the package insert, enclosed herein as Attachment B. The amino acid sequence of ecallantide (see SEQ ID NO: 2 of U.S. Patent No. 7,276,480, provided as Attachment C) is as follows:

Attorney Docket No.: D2033-7060US/10280-096US1

In re U.S. Patent No.: 5,795,865

Issued: August 18, 1998

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Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

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# (2) Federal Statute Governing Regulatory Approval of the Approved Product [1.740(a)(2)]

The approved product, KALBITOR®, was subject to regulatory review under § 505(i) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 355(i)) and § 351(a) of the Public Health Service Act (42 U.S.C. § 262(a)).

### (3) Date of Approval for Commercial Marketing [1.740(a)(3)]

The approved product, KALBITOR®, received permission for commercial marketing or use under Section 351 (a) of the Public Health Service Act on December 1, 2009. A copy of the FDA letter issuing Biologics License No. 1789 is attached (Attachment D).

# (4) Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [1.740(a)(4)]

The only active ingredient in KALBITOR® is ecallantide which, on information and belief, has not been approved for commercial marketing or use under the Public Health Service Act, the Virus-Serum-Toxin Act or the Federal Food, Drug and Cosmetic Act prior to the issuance of Biologics License No. 1789 by the Food and Drug Administration on December 1, 2009. A copy of the package insert describing the approved product is attached (Attachment B).

# (5) Statement Regarding Timeliness of Submission of Patent Term Extension Request [1.740(a)(5)]

This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the permitted 60-day period pursuant to 37 C.F.R. § 1.720(f). The last day on which this application can be submitted is January 29, 2010.

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# (6) Complete Identification of the Patent for Which Extension Is Being Sought [1.740(a)(6)]

The complete identification of the patent for which a term extension is being sought is as follows:

Inventors:

William Markland and Robert Charles Ladner

Patent No.:

5,795,865

Filing Date:

PCT filed January 11, 1995

Issue Date:

August 18, 1998

Expiration Date:

August 18, 2015

Attorney Docket No.: D2033-7060US/10280-096US1

In re U.S. Patent No.: 5,795,865

Issued: August 18, 1998

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Charles Ladner

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## (7) Copies of the Patent for Which an Extension is Being Sought [1.740(a)(7)]

A copy of U.S. Patent No.: 5,795,865 is provided as Attachment E.

# (8) Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payments, or Reexamination Certificate [1.740(a)(8)]

- (a) U.S. Patent No.: 5,795,865 was subject to a terminal disclaimer over any patent granted on U.S. Application Serial No.: 08/208,264, filed March 10, 1994. (See Attachment F);
- (b) A Certificate of Correction was entered for U.S. Patent No.: 5,795,865. A copy of a Certificate of Correction and a Notice Regarding Request for Certificate of Correction is attached (Attachment G);
- (c) The first maintenance fee for U.S. Patent No.: 5,795,865, due August 19, 2002, was paid. The second maintenance fee, due August 18, 2006, was paid. A copy of a record of maintenance fee payments under 35 U.S.C. § 41(b) is attached (Attachment H). The next maintenance fee will be due August 18, 2010.
- (d) U.S. Patent No.: 5,795,865 has not been the subject of a reexamination proceeding.

## (9) Statement Regarding Patent Claims Relative to Approved Product [1.740(a)(9)]

- (a) The following claims of U.S. Patent No. 5,795,865 claim the approved product, KALBITOR® (ecallantide), or a method of using the approved product: claims 1, 2, 5, 6 and 7.
- (b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows that the above listed claims of U.S. Patent No.

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5,795,865 claim the approved product, KALBITOR® (ecallantide) or an approved method for using KALBITOR® (ecallantide).

(1) Description of the approved product and its approved method of use

The approved product is described as follows in the package insert for KALBITOR®, a copy of which is provided as Attachment B: "KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein."

The amino acid sequence of ecallantide (see SEQ ID NO: 2 of U.S. Patent No. 7,276,480, provided as Attachment C) is as follows:

A portion of the amino acid sequence of ecallantide, beginning with the Met at residue 3 of the ecallantide amino acid sequence provided above, is a non-naturally occurring Kunitz domain obtained by semi-random mutagenesis of a naturally occurring Kunitz domain, see U.S. Patent No. 7,206,480 (Attachment C).

KALBITOR® is approved for the treatment of acute attacks of hereditary angioedema (HAE) in patients. Hereditary angioedema is described in the package insert for KALBITOR® as follows:

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on chromosome 11q and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade

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involved in both the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kininogen to bradykinin by protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Brandykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

(2) Description of claims 1, 2, 5, 6 and 7 and comparison to the active ingredient of KALBITOR® and its approved method of use

The following description demonstrates the manner in which at least one claim of U.S. Patent No. 5,795,865 reads on the approved product and at least one claim of U.S. Patent No. 5,795,865 reads on a method of using approved product.

As will be seen in the discussion below, certain claims of U.S. Patent No. 5,795,865, refer to the amino acid sequence numbering system of bovine pancreatic trypsin inhibitor (BPTI). An alignment of the relevant portion of the amino acid sequence of ecallantide with the amino acid sequence of bovine pancreatic trypsin inhibitor (BPTI) (as shown in Table 2 of U.S. Patent No.: 5,795,865, see Attachment E) is provided in Attachment I.

(c) Claim 1 of U.S. Patent No. 5,795,865 reads on the approved product. Claim 1 is set out in the left hand column of the table immediately below. The approved product is described in the right hand column and compared with the claim. As is shown, the approved product meets all of the limitations of the claim and the claim covers the approved product.

: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

In re U.S. Patent No.: 5,795,865 Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

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Claim	n 1		KALBITOR® (ecallantide)
non-nat eac corres	A kallikrein inhibiting protein which comprises a non-naturally occurring Kunitz domain, wherein, at each of the residues of said domain corresponding to the below identified residues of BPTI, one of the following allowed amino acids is found:		A "kallikrein inhibiting protein" is defined at column 4, lines 38-40 of U.S. Patent No. 5,795,865 as "one that binds and inhibits a specified kallikrein with K <sub>i</sub> of about 20nM or less." Ecallantide meets this limitation as it binds to and inhibits plasma kallikrein with a K <sub>i</sub> of 25 pM. (Attachment B).  Ecallantide comprises a non-naturally occurring Kunitz domain, see section (9)(b)(1) above.  As is discussed below, ecallantide meets each of the sequence limitations of the claim:
B	PTI	Allowed Amino Acid	
Resi	idue#		
10		Asp, Glu, Ala, Gly, Ser, Thr	The amino acid residue in ecallantide which corresponds to this position is Asp, thus this limitation of the claim is met.
11		Asp, Gly, Ser, Val, Glu, Leu, Met, Asn, Ile, Ala, Thr	The amino acid residue in ecallantide which corresponds to this position is Asp, thus this limitation of the claim is met.
12		Gly, and, if residue 14 or 38 is not Cys, any conservative or semi- conservative substitution for a "normal" conformation Gly as defined in Table 9	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this limitation of the claim is met.
13		Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys, Gln	The amino acid residue in ecallantide which corresponds to this position is Pro, thus this limitation of the claim is met.
14		Cys, and, if residue 38 is not Cys, any conservative or semi-	The amino acid residue in ecallantide which corresponds to this position is Cys, thus this

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	Conservative substitution for Cys	limitation of the claim is met.
15	Arg, Lys, Ala, Ser, Gly, Met, Asn, Gln	The amino acid residue in ecallantide which corresponds to this position is Arg, thus this limitation of the claim is met.
16	Ala, Gly, Ser, Asp, Asn	The amino acid residue in ecallantide which corresponds to this position is Ala, thus this limitation of the claim is met.
17	Ala, Asn, Ser, Ile, Gly, Val, Gln, Thr	The amino acid residue in ecallantide which corresponds to this position is Ala, thus this limitation of the claim is met.
18	His, Leu, Gln, Ala	The amino acid residue in ecallantide which corresponds to this position is His, thus this limitation of the claim is met.
19	Pro, Gln, Leu, Asn, Ile	The amino acid residue in ecallantide which corresponds to this position is Pro, thus this limitation of the claim is met.
20	Arg, Leu, Ala, Ser, Lys, Gln, Val	The amino acid residue in ecallantide which corresponds to this position is Arg, thus this limitation of the claim is met.
21	Trp, Phe, Tyr, His, Ile	The amino acid residue in ecallantide which corresponds to this position is Trp, thus this limitation of the claim is met.
31	Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile, Thr	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.
32	Glu, Gln, Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly, Val	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.
33	Phe, Tyr	The amino acid residue in ecallantide which corresponds to this position is Phe, thus this limitation of the claim is met.
34	Ser, Thr, Ile, Val, Ala, Asn, Gly, Leu	The amino acid residue in ecallantide which corresponds to this position is Ile, thus this limitation of the claim is met.
35	Tyr, Trp, Phe	The amino acid residue in ecallantide which corresponds to this position is Tyr, thus this limitation of the claim is met.
36	Gly, Ser, Ala	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this

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		limitation of the claim is met
37	Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this limitation of the claim is met.
38	Cys, and, if residue 14 is not Cys, any conservative or semiconservative substitution for Cys	The amino acid residue in ecallantide which corresponds to this position is Cys, thus this limitation of the claim is met.
39	Gly, Glu, Ala, Ser, Asp.	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.

(d) Claim 2 of U.S. Patent No. 5,795,865 reads on the approved use of the approved product. Claim 2 is set out in the left hand column of the table immediately below. The approved use of the approved product is described in the right hand column and compared with the claim. As is shown, the approved use of the approved product meets all of the limitations of the claim and the claim covers the approved use of the approved product.

Claim 2	The approved use
A method of treating a disorder attributable to excessive kallikrein activity which comprises	The approved use is the treatment of HAE. As discussed above in the excerpt from the package insert, HAE is a disorder attributable to excessive kallikrein activity.
administering, to a human or animal subject who would benefit therefrom,	KALBITOR® has been approved to treat acute attacks of hereditary angioedema (HAE) in patients. Thus, it is administered to a subject who would benefit therefrom.
a kallikrein-inhibitory amount of the protein of claim 1.	As discussed for claim 1 above, ecallantide meets all of the structural and functional limitations of the kallikrein inhibiting

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	protein of claim 1.

(e) Claim 5 of U.S. Patent No. 5,795,865 reads on the approved product. Claim 5 is set out in the left hand column of the table immediately below. The approved product is described in the right hand column and compared with the claim. As is shown, the approved product meets all of the limitations of the claim and the claim covers the approved product.

Claim 5		KALBITOR®
non-naturally at each of the	nhibiting protein which comprises a coccurring Kunitz domain, wherein, residues corresponding to the below idues, one of the following allowed s found:	A "kallikrein inhibiting protein" is defined at column 4, lines 38-40 of U.S. Patent No. 5,795,865 as "one that binds and inhibits a specified kallikrein with K <sub>i</sub> of about 20nM or less." Ecallantide meets this limitation as it binds to and inhibits plasma kallikrein with a K <sub>i</sub> of 25 pM. (Attachment B).  Ecallantide comprises a non-naturally occurring Kunitz domain, see section (9)(b)(1) above.  As is discussed below, ecallantide meets each of the sequence limitations of the claim:
BPTI	Allowed Amino Acid	
Residue #		
10	Asp, Glu, Ala, Gly, Ser, Thr	The amino acid residue in ecallantide which corresponds to this position is Asp, thus this limitation of the claim is met.
11	Asp, Gly, Ser, Val, Glu, Leu, Met	The amino acid residue in ecallantide which corresponds to this position is Asp, thus this

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		limitation of the claim is met.
12	Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this limitation of the claim is met.
13	Arg, His, Pro, Asn, Ser	The amino acid residue in ecallantide which corresponds to this position is Pro, thus this limitation of the claim is met.
14	Cys, and, if residue 38 is not Cys, any conservative or semiconservative substitution for Cys	The amino acid residue in ecallantide which corresponds to this position is Cys, thus this limitation of the claim is met.
15	Arg, Lys	The amino acid residue in ecallantide which corresponds to this position is Arg, thus this limitation of the claim is met.
16	Ala, Gly	The amino acid residue in ecallantide which corresponds to this position is Ala, thus this limitation of the claim is met.
17	Ala, Asn, Ser, Ile	The amino acid residue in ecallantide which corresponds to this position is Ala, thus this limitation of the claim is met.
18	His, Leu, Gln	The amino acid residue in ecallantide which corresponds to this position is His, thus this limitation of the claim is met.
19	Pro, Gln, Leu	The amino acid residue in ecallantide which corresponds to this position is Pro, thus this limitation of the claim is met.
20	Arg, Leu, Ala, Ser, Lys, Gln, Val	The amino acid residue in ecallantide which corresponds to this position is Arg, thus this limitation of the claim is met.
21	Trp, Phe	The amino acid residue in ecallantide which corresponds to this position is Trp, thus this limitation of the claim is met.
31	Glu	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.

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32	Glu, Gln	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.
33	Phe	The amino acid residue in ecallantide which corresponds to this position is Phe, thus this limitation of the claim is met.
34	Ser, Thr, Ile	The amino acid residue in ecallantide which corresponds to this position is Ile, thus this limitation of the claim is met.
35	Tyr	The amino acid residue in ecallantide which corresponds to this position is Tyr, thus this limitation of the claim is met.
36	Gly, Ser, Ala	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this limitation of the claim is met
37	Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9	The amino acid residue in ecallantide which corresponds to this position is Gly, thus this limitation of the claim is met.
38	Cys, and, if residue 14 is not Cys, any conservative or semi-conservative substitution for Cys	The amino acid residue in ecallantide which corresponds to this position is Cys, thus this limitation of the claim is met.
39	Gly, Glu, Ala.	The amino acid residue in ecallantide which corresponds to this position is Glu, thus this limitation of the claim is met.

(f) Claim 6 reads as follows: The protein of claim 2 wherein, the Kunitz domain is further characterized as follows:

- 10 Asp, Glu
- 11 Asp, Gly, Ser, Val
- 12 Gly
- 14 Cys
- 20 Arg
- 36 Gly

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37 Gly

38 Cys.

Claim 6 of U.S. Patent No. 5,795,865 reads on and covers ecallantide. As discussed above, ecallantide meets all of the limitations of the base claim. Each of the further limitations of claim 6 is met as well: at position 10, the amino acid residue of ecallantide is Asp; at position 11, the amino acid residue of ecallantide is Asp; at position 12, the amino acid residue of ecallantide is Gly; at position 14, the amino acid residue of ecallantide is Cys; at position 20, the amino acid residue of ecallantide is Arg; at position 36, the amino acid residue of ecallantide is Gly; at position 37, the amino acid residue of ecallantide is Gly; and at position 38, the amino acid residue of ecallantide is Cys.

(g) Claim 7 of U.S. Patent No. 5,795,865 reads on the approved use of the approved product. Claim 7 is set out in the left hand column of the table immediately below. The approved use of the approved product is described in the right hand column and compared with the claim. As is shown, the approved use of the approved product meets all of the limitations of the claim and the claim covers the approved use of the approved product.

Claim 7	The approved use
A method of treating a disorder attributable to excessive kallikrein activity which comprises	The approved use is the treatment of HAE. As discussed above in the excerpt from the package insert, HAE is a disorder attributable to excessive kallikrein activity.
administering, to a human or animal subject who would benefit therefrom,	KALBITOR® has been approved to treat acute attacks of hereditary angioedema (HAE) in patients. Thus, it is administered to a subject who would benefit therefrom.
a kallikrein-inhibitory amount of the protein of claim 5.	As discussed for claim 5 above, ecallantide meets all of the structural and functional

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	limitations of the kallikrein inhibiting protein of claim 5.

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Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

## (10) Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [1.740(a)(10)]

The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

#### (a) Patent Issue Date:

U.S. Patent No. 5,795,865 issued on August 18, 1998.

## (b) IND Effective Date and IND number [35 U.S.C. §156(g)(1)(B)(i); 37 C.F.R. §1.740(a)(10)(i)(A)]

A first IND was by submitted by Dyax Corp. to the FDA and received by the FDA on January 11, 2002. It was assigned number BB-IND#10232. A copy of the letter from the FDA to Dyax Corp. providing the IND number and showing the date of receipt by the FDA of the first IND is provided in Attachment J1. BB-IND#10232 was concerned with the use of ecallantide in patients undergoing cardiopulmonary bypass procedures associated with cardiothoracic surgery (CTS). In a telephone conference between Dyax Corp. and the FDA on February 8, 2002, the FDA indicated that clinical trials under BB-IND#10232 could be initiated. A copy of "Record of Contact" memorializing that telephone conference made by Dyax Corp. is provided in Attachment J2. This exemption became effective February 8, 2002.

A second IND was submitted by Dyax Corp. to the FDA and received by the FDA on May 1, 2002. It was assigned number BB-IND#10426. A copy of the letter from the FDA to Dyax Corp. providing the IND number and showing the date of receipt by the FDA of the IND is provided in Attachment K. BB-IND#10426 was concerned with the use of ecallantide to treat angioedema, in particular hereditary angioedema (HAE). BB-IND#10426 cross referenced the earlier filed BB-IND#10232 and relied on chemistry,

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manufacture and control (CMC) and pre-clinical studies data provided in the earlier filed IND. In a telephone conference between Dyax Corp. and the FDA on May 30, 2002, the FDA indicated that clinical trials under BB-IND#10426 could be initiated. A copy of a letter from Dyax Corp. to the Center for Biologics Evaluation and Research summarizing that call is provided in Attachment L. This exemption became effective May 30, 2002.

Both INDs were transferred to the Center for Drug Evaluation and Research in 2003 when recombinant therapeutic proteins were transferred by the FDA.

In a communication dated June 12, 2008, the earlier IND, BB-IND#10232, was conveyed to Cubist Pharmaceuticals effective as of June 16, 2008. A copy of the communication dated June 12, 2008 from Dyax Corp. to the Center for Drug Evaluation and Research is provided in Attachment M.

By a communication dated June 13, 2008, BB-IND#10426 was amended by addition of the data it relied on from the earlier filed IND. A copy of the communication from Dyax Corp. to the Center for Drug Evaluation and Research is provided in Attachment N.

Thus, as set out above, the date that an exemption under §505(i) of the Federal Food, Drug and Cosmetic Act became effective (i.e., the date that an investigational new drug application (IND) became effective for KALBITOR®) was February 8, 2002.

# (c) BLA Submission Date [35 U.S.C. §156(g)(1)(B)(i); 37 C.F.R. §1.740(a)(10)(i)(B)]

The BLA was submitted on a rolling basis. Accordingly, the initial portion of the BLA was submitted by Dyax to the FDA on December 31, 2007. The final portion was submitted on September 23, 2008. This date is used in the calculations provided herein. The BLA was assigned number BL 125277/0. A copy of the letter from the FDA acknowledging receipt of the BLA application is provided as Attachment O.

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### (d) BLA Issue Date [35 U.S.C. §156(g)(1)(B)(ii); 37 C.F.R. §1.740(a)(10)(i)(C)]

The FDA approved BLA 125277/0 authorizing the marketing of KALBITOR® on December 1, 2009. KALBITOR® was approved under the Department of Health and Human Services (DHHS) U.S. License No.: 1789. A copy of the approval letter from the FDA is provided as Attachment D.

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# (11) Summary of Significant Events During Regulatory Review Period [1.740(a)(11)]

A brief description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to KALBITOR® and the dates applicable to these significant activities are set forth in a chronology of events provided below.

	Brief Description of Significant Activities During Regulatory Review Period for DX-88 (ecallantide) for 3 associated applications: 1) BB IND 10232 for CTS Indication (HAE IND cross-referred to this IND for CMC and Nonclinical Information); 2) BB IND 10426 for HAE Indication; 3) BLA 125277	
Date	Significant Activity	Application
10-Jan-02	BB-IND10232 submitted to FDA	BB-IND 10232
11-Jan-02	BB-IND10232 received by FDA	BB-IND 10232
8-Feb-02	BB-IND10232 in effect	BB-IND 10232
30-Apr-02	BB IND 10426 submitted to FDA	BB-IND 10426
1-May-02	BB IND 10426 received by FDA	BB-IND 10426
30-May-02	Submission: Response to 29 May clinical teleconference	BB-IND 10426
	BB-IND 10426 in effect	
31-May-02	Submission: Response to 30 May teleconference	BB-IND 10426
21-Nov-02	FDA Orphan Designation for HAE and AAE (Designation 02-1608)	BB-IND 10426
7-Feb-03	Submission: IND Annual Report	BB-IND 10232
29-May-03	Submission: IND Annual Report	BB-IND 10426
20-Jun-03	FDA Communication regarding transfer of biologic therapeutic products from CBER to CDER.	BB-IND 10232
26-Jun-03	Submission: Reformatted Fast Track request	BB-IND 10426
5-Aug-03	Communication from FDA: Fast Track denied	BB-IND 10426
4-Mar-04	Submission: IND Annual Report	BB-IND 10232
8-Apr-04	Teleconference with FDA regarding protocol	BB-IND 10426
13-May-04	Submission: IND Annual Report	BB-IND 10426
10-Jun-04	Call to FDA regarding Dyax press release describing topline results of	BB-IND 10426

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Date	Significant Activity	Application
	EDEMA1	
6-Jul-04	Submission: Revised IB and informed consent	BB-IND 10426
29-Jul-04	Call with FDA to discuss EDEMA2 treatments per patient	BB-IND 10426
31-Jul-04	Call to FDA regarding Fast Track denial and request for re-examination	BB-IND 10426
9-Aug-04	Submission: request for EOP2 meeting	BB-IND 10426
11-Aug-04	Submission: Fast Track request resubmitted	BB-IND 10426
7-Sep-04	Submission: Request for EOP2/Pre-BLA meeting	BB-IND 10426
21-Sep-04	FDA phoned with suggestion on Fast Track designation	BB-IND 10426
24-Sep-04	Submission: Fast Track request additional information	BB-IND 10426
18-Oct-04	EOP2 meeting via teleconference	BB-IND 10426
22-Oct-04	Call with FDA to discuss topics from EOP2 Meeting	BB-IND 10426
30-Nov-04	Pivotal trial design telecon with FDA	BB-IND 10426
8-Dec-04	Submission: Response to comments at EOP2 telecon	BB-IND 10426
10-Feb-05	Teleconference with FDA to discuss design of pivotal study	BB-IND 10426
28-Feb-05	Call to FDA requesting additional feedback on pivotal trial design	BB-IND 10426
1-Mar-05	Call to FDA to discuss EOP2 meeting delay due to further discussion on integrating intravenous and subcutaneous clinical programs.	BB-IND 10426
3-Mar-05	Call from FDA to discuss plans for a meeting between Office of Orphan Drugs and ODE VI	BB-IND 10426
4-Mar-05	Call to FDA to discuss recruitment in ongoing EDEMA2 study.	BB-IND 10426
4-Mar-05	Orphan Office called to discuss meeting with ODE VI reviewers	BB-IND 10426
10-Mar-05	Dyax called FDA to discuss the primary endpoint for EDEMA3	BB-IND 10426
30-Mar-05	Submission: IND Annual Report	BB-IND 10232
29-Apr-05	Teleconference discussing endpoint for EDEMA3	BB-IND 10426
26-May-05	Submission: IND Annual Report	BB-IND 10426
14-Jul-05	EOP2 meeting	BB-IND 10426
23-Sep-05	Call with FDA to discuss IND reviews following FDA reorganization	BB-IND 10232
3-Oct-05	FDA Communication: Comments to EDEMA3 protocol (eg DX-88/14)	BB-IND 10426
19-Oct-05	Submission: Response to FDA comments for EDEMA3 study	BB-IND 10426
20-Oct-05	Submission: DMSB report	BB-IND 10426

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Date	Significant Activity	Application
27-Dec-05	Submission: Information supporting the proposed modification of the Fast	BB-IND 10426
	Track objective	BB 114B 10420
10-Feb-06	Submission: New Fast Track request	BB-IND 10426
17-Mar-06	Submission: IND Annual Report	BB-IND 10232
30-May-06	Submission: IND Annual Report	BB-IND 10426
29-Aug-06	Type B meeting	BB-IND 10426
3-Oct-06	Meeting: Type B meeting	BB-IND 10232
18-Oct-06	Submission: Request for Fast Track Designation	BB-IND 10426
20-Nov-06	FDA Letter: Fast Track approval	BB-IND 10426
9-Jan-07	FDA sent comments to CTS clinical protocol DX88/16	BB-IND 10232
11-Jan-07	FDA Communication: Meeting minutes from 13 Dec 06 meeting	BB-IND 10426
17-Jan-07	Type A Meeting via teleconference regarding SPA for EDEMA4	BB-IND 10426
13-Feb-07	Submission: Responses to FDA comments to CTS clinical protocol DX88/16	BB-IND 10232
9-Apr-07	Submission: IND Annual Report	BB-IND 10232
29-May-07	Submission: IND Annual Report	BB-IND 10426
13-Jun-07	Submission: Type C Briefing Package for 16 July 2007 meeting to discuss Filability based on positive Phase 3 (EDEMA3) results	BB-IND 10426
12-Jul-07	FDA Communication: Draft Responses to Questions for 16 July 07 meeting regarding filing on EDEMA3 package	BB-IND 10426
1-Aug-07	Submission: preBLA Type B Meeting Request	BB-IND 10426
9-Aug-07	Submission: Proprietary name review request	BB-IND 10426
1-Oct-07	Submission: Pre-BLA Briefing Book for the October 30th, 2007 meeting	BB-IND 10426
24-Oct-07	FDA Letter: SPA Agreement	BB-IND 10426
30-Oct-07	Pre-BLA meeting	BB-IND 10426
19-Nov-07	Email to FDA requesting BLA number	BB-IND 10426
19-Nov-07	Email submitting Dyax information to FDA to obtain BLA	BB-IND 10426
20-Nov-07	FDA Letter: Assignment of BLA number	BB-IND 10426
20-Nov-07	Assignment and confirmation of BLA from FDA	BB-IND 10426
20-Dec-07	Submission: Rolling Review Request	BB-IND 10426
31-Dec-07	Submission: CMC rolling submission	BLA125277

In re U.S. Patent No.: 5,795,865 Issued: August 18, 1998 Attorney Docket No.: D2033-7060US/10280-096US1

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Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

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Date	Significant Activity	Application
18-Jan-08	FDA Communication: Acceptance of submission of rolling sections of BLA	BB-IND 10426
27-Mar-08	Submission: Nonclinical rolling submission	BLA125277
9-Apr-08	Submission: IND Annual Report	BB-IND 10232
7-May-08	Submission: IND Annual Report	BB-IND 10426
12-Jun-08	Submission: Notification to FDA that BB-IND 10232 was transferred to Cubist Pharmaceuticals, effective 16June2008	BB-IND 10232
13-Jun-08	Submission: Copied to BB-IND 10426 the CMC and nonclinical submissions that had previously been submitted to BB-IND 10232. The submission ensured that from this point forward BB-IND 10426 no longer relied on BB-IND 10232 for CMC and nonclinical.	BB-IND 10426
23-Sep-08	Submission: Original BLA submission completed (starting PDUFA clock)	BLA125277
10-Oct-08	Submission: Response to Office of Compliance questions	BLA125277
24-Oct-08	Teleconference regarding Pre-Approval Inspection of drug substance facility	BLA125277
17-Nov-08	Teleconference regarding Pre-Approval Inspection of drug substance facility	BLA125277
20-Nov-08	FDA Letter: Filing of the BLA including initial review comments/questions	BLA125277
8-Jan-09	Teleconference regarding advisory committee topics	BLA125277
25-Mar-09	FDA Action Letter: Complete Response	BLA125277
23-Apr-09	Submission: IND Annual Report	BB-IND 10426
31-May-09	Submission: BLA resubmission	BLA125277
5-Jun-09	FDA Letter: Acknowledgment of BLA resubmission receipt	BLA125277
5-Aug-09	FDA fax with vial/carton comments	BLA125277
12-Aug-09	Submission: Response to vial/carton label comments	BLA125277
4-Sep-09	Communication from FDA indicating preliminary acceptability of tradename	BLA125277
7-Oct-09	Teleconference regarding proposed REMS	BLA125277
16-Oct-09	FDA Letter regarding REMS requirements	BLA125277
16-Oct-09	FDA Communication with labeling comments	BLA125277
20-Oct-09	Teleconference regarding labeling	BLA125277
26-Oct-09	Submission: Proposed REMS with revisions per FDA Communication of	BLA125277

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Date	Significant Activity	Application
	16 October 2009	
29-Oct-09	FDA Letter:B36 BLA acknowledgement	BLA125277
19-Nov-09	FDA Communication with REMS comments	BLA125277
20-Nov-09	FDA Communication with labeling comments	BLA125277
24-Nov-09	Teleconference regarding post marketing requirements	BLA125277
1-Dec-09	FDA Action Letter: BLA Approval	BLA125277

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# (12) Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. §1.740(a)(12)]

- (i) Applicant is of the opinion that U.S. Patent No. 5,795,865 is eligible for extension of the patent term under 35 U.S.C. § 156 of 1645 days and should be extended until February 18, 2020. It satisfies all requirements for such extension including:
- (a) 35 U.S.C. § 156(a) U.S. Patent No. 5,796,865 claims ecallantide, a kallikrein inhibiting protein (the active ingredient in KALBITOR®), and methods of using the active ingredient.
- (b) 35 U.S.C. § 156(a)(1) U.S. Patent No. 5,796,865 has not expired before submission of this application.
- (c) 35 U.S.C. § 156(a)(2) The term of U.S. Patent No. 5,795,865 has never been extended under 35 U.S.C. § 156(e)(1).
- (d) 35 U.S.C. § 156(a)(3) The application for patent term extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.
- (e) 35 U.S.C. § 156(a)(4) The product KALBITOR® has been subject to a regulatory review period before its commercial marketing or use.
- (f) 35 U.S.C. § 156(a)(5)(A) The commercial marketing or use of the product KALBITOR® after the regulatory review period is the first permitted commercial marketing or use under the provisions of § 351 (a) of the Public Health Service Act under which such regulatory review period occurred.
- (g) 35 U.S.C. § 156(c)(4) No other patent has been extended for the same regulatory review period for the product KALBITOR®.
- (h) This application is being submitted within 60 days of regulatory agency approval.
- (i) This application otherwise complies with all requirements of 35 U.S.C. § 156 and all applicable rules and procedures.

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(12)(ii) Applicant respectfully submits that the length of the extension of patent term for U.S. Patent No. 5,795,865 is 1645 days pursuant to 35 U.S.C. § 156(c).

The length of the extension was determined pursuant to 37 C.F.R. § 1.775 as follows (the remainder of this section (12)(ii) is numbered so as to correspond to the numbering in 37 C.F.R. § 1.775 ):

- (c) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) is a total of 2855 days, which is the sum of (1) and (2) below:
- (1) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), which is the number of days in the period beginning on the date the exemption became effective (February 8, 2002) and ending on the date an application was initially submitted (September 23, 2008), which is 2420 days; and
- (2) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), which is the number of days in the period beginning on the date the application was initially submitted (September 23, 2008) and ending on the date such application was approved (December 1, 2009), which is 435 days.
- (d) The term of the patent as extended for a human drug, antibiotic drug or human biological product is determined by:
- (1) Subtracting from the number of days determined to be in the regulatory review period, which is 2855:
- (i) The number of days in the regulatory review period which were on or before the date on which the patent issued (August 18, 1998) which is zero (0) days; and
- (ii) The number of days in the period of (c)(1) and (c)(2) above during which applicant did not act with due diligence, which is zero (0) days; and (iii) One-half the number of days determined in

subparagraph (c)(1) above after that period is reduced by subparagraph (d)(1)(i) and (d)(1)(ii) which, is (2420-0-0)/2, or 1210 days.

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Thus, the number of days determined in subparagraph (c) above (2855) is reduced by 1210 days, for a total of 1645 days;

- (2) Adding the number of days as determined in subparagraph (d)(1), (1645 days), to the original term of the patent (August 18, 2015) which results in the date of February 18, 2020.
- (3) By adding fourteen (14) years to the date of issuance of the Biologics License (December 1, 2009) which results in the date of December 1, 2023;
- (4) By comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) and selecting the earlier, which is February 18, 2020;
- (5) (i) Since U.S. Patent No. 5,795,865 issued after September 24, 1984, by adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer, which is August 18, 2020; and (ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date, which is February 18, 2020.

Thus, the patent is entitled to extension until February 18, 2020.

### (13) Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, e.g., as that duty is defined in 37 C.F.R. § 1.765.

#### (14) Applicable Fee [1.740(a)(14)]

The prescribed fee for receiving and acting upon this application is attached as a check in the amount of \$1,120.00. The Director is authorized to charge any additional

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fees required by this application to Deposit Account No. 50/2762, referencing attorney docket number D2033-7060US.

### (15) Name and Address for Correspondence [1.740(a)(15)]

All correspondence and inquiries may be directed to the undersigned, whose address, telephone number and fax number are as follows:

Laurie Butler Lawrence Lando & Anastasi, LLP One Main Street Cambridge, MA 02142 Phone: 617-395-7000

Fax: 617-395-7070

Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof (Attachment P) in compliance with 37 C.F.R. § 1.740(b).

Respectfully submitted,

Laurie Butler Lawrence, Reg. No. 46,593

LANDO & ANASTASI, LLP

One Main Street

Cambridge, Massachusetts 02142

United States of America Telephone: 617-395-7000 Facsimile: 617-395-7070

Attachments:

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

Power of Attorney (Attachment A)

Package Insert for KALBITOR® (Attachment B)

U.S. Patent No. 7,276,480, (Attachment C)

Biologics License Approval Letter including enclosures (Attachment D)

U.S. Patent No. 5,795,865 (Attachment E)

Terminal Disclaimer (Attachment F)

Certificate of Correction (Attachment G)

Maintenance Fee Statement (Attachment H)

Alignment of the Kunitz domain of ecallantide and bovine trypsin protease inhibitor (Attachment I)

Letter from FDA acknowledging receipt of the first IND (Attachment J1)

Contact Report for DYAX-FDA Teleconference of February 8, 2002 (Attachment

Letter from FDA acknowledging receipt of the second IND (Attachment K)

Letter from Dyax to the Center for Biologic Evaluation and Research dated May

31, 2002 which summarized the May 30, 2002 telephone conference (Attachment L)

Communication dated June 12, 2008 from Dyax Corp. to the Center for Drug

Evaluation and Research discussing conveyance of BB-IND#10232 to Cubist

Pharmaceuticals (Attachment M)

Communication from Dyax Corp. to the Center for Drug Evaluation and Research dated June 13, 2008, in which BB-IND#10426 was amended (Attachment N)

Letter from FDA acknowledging receipt of the final submission of the BLA (Attachment O)

Certification of Copies of Application Papers (Attachment P)

J2)

In re U.S. Patent No.: 5,795,865 Issued: August 18, 1998

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Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

Attorney Docket No.: D2033-7060US/10280-096US1

ANALOGUES THEREOF

Attachment A

Power of Attorney

### **ATTACHMENT A**

Docket No.: D2033-9000

#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

# REVOCATION OF PRIOR POWERS OF ATTORNEY and NEW POWER OF ATTORNEY

Sir:

The undersigned, Dyax Corp., a Delaware Corporation, assignee of the entire right, title and interest for all of the patents and patent applications identified in the attached Schedule A, hereby revokes all previous powers of attorney or authorizations of agent given in the identified patents and patent applications and in any divisional, continuing, substitute, renewal, reexamination, or reissue applications thereof, and appoints all practitioners of Lowrie, Lando & Anastasi, LLP associated with Customer Number:

### 37462

as assignee's attorneys or agents with full power of substitution to take any and all action necessary with regard to the identified patents and patent applications, and with regard to any divisional, continuing, substitute, renewal or reissue applications thereof.

Please address all telephone calls to Laurie Butler Lawrence at telephone no. (617) 395-7000.

Please forward all correspondence to the correspondence address associated with Customer Number:

37462

Dyax Ørp.

Name: Ivana Magovcevic Liebisch, Ph.D. ID

Title: General Counsel and

Executive Vice President of Administration

### **ASSIGNEE CERTIFICATION**

Attached to this power is a Certificate Under 37 CFR 3.73(b).

Dated: (h) + WX

/Laurie Butler Lawrence/
Laurie Butler Lawrence, Reg. No. 46,593
LOWRIE, LANDO & ANASTASI, LLP
Riverfront Office Park
One Main Street
Cambridge, MA 02142
(617) 395-7000

### [CLICK HERE AND TYPE COMPANY NAME]

TO:	FROM:	
Mary Till	Laurie Butler Lawrence	
COMPANY: Lando & Anastasi	DATE: 5/5/2010	
рах number: 571-273-7755	TOTAL NO. OF PAGES INCLUDING COVER:	
PHONE NUMBER:	SENDER'S REFERENCE NUMBER:	
RE: Statement under 3.73(b)	YOUR REFERENCE NUMBER: D2033-7060US (Patent No.: 5,795,865)	
☑ URGENT ☐ FOR REVIEW	☐ PLEASE COMMENT ☐ PLEASE REPLY ☐ PLEASE RECYCLE	

[CLICK HERE AND TYPE RETURN ADDRESS]

PTO/SB/96 (12-07)

Approved for use through 12/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE der the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)				
Applicant/Patent Owner: Markland et al.	· ·			
Application No./Patent No.: 5,795,865	_ Filed/Issue Date: _08/18/1998			
Entitled: KALLIKREIN-BINDING "KUNITZ DOMAIN" PROTEINS AND ANALOGUES THEREOF				
<u>Dyax Corp</u> (Name of Assignee)	(Type of Assignee, e.g., corporation, partnership,	university, government agency, etc.)		
states that it is:  1.  the assignee of the entire right, title, and interest.	est; or			
2. an assignee of less than the entire right, title at (The extent (by percentage) of its ownership in	and interest interest is%)			
in the patent application/patent identified above by v	virtue of elther:			
A An assignment from the Inventor(s) of the pate in the United States Patent and Trademark Of thereof is attached.	ent application/patent identified above. The a flice at Reel <u>8321</u> , Frame <u>0061-65</u>	assignment was recorded , or for which a copy		
OR  B. A chain of title from the inventor(s), of the pate	ent application/patent identified above, to the	current assignee as follows:		
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[NOTE: A separate copy ( <i>i.e.</i> , a true copy of the Division in accordance with 37 CFR Part 3 302.08]	e original assignment document(s)) must be 3, to record the assignment in the records of	submitted to Assignment the USPTO. <u>See</u> MPEP		
The undersigned (whose title is supplied below) is a	authorized to act on behalf of the assignee.			
/Laurie Butler Lawren		03/27/2008		
Signature		Date		
Laurie Butler Lawrence, Reg.	No. 46.593	617-395-7000		
Printed or Typed Nar		Telephone Number		
Attorney Title				

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief information Officer, U.S. Patent and Trademerk Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND PEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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# SCHEDULE A

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In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

Attachment B

Package Insert for KALBITOR®

# ATTACHMENT B

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use
KALBITOR® safely and effectively. See full prescribing information for
KALBITOR.

KALBITOR (ecallantide) injection, for subcutaneous use Initial U.S. Approval: 2009

### **WARNING: ANAPHYLAXIS**

See full prescribing information for complete boxed warning

Anaphylaxis has been reported after administration of KALBITOR®. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)].

### ---INDICATIONS AND USAGE--

 KALBITOR is a plasma kallikrein inhibitor indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older. (1)

### --DOSAGE AND ADMINISTRATION----

- 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If an attack persists, an additional dose of 30 mg may be administered within a 24 hour period. (2.1)
  - KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. (2.2).

### ---DOSAGE FORMS AND STRENGTHS--

Single use glass vial containing 10 mg/mL of ecallantide as a solution for injection. (3)

#### -CONTRAINDICATIONS---

 Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. (4)

### ------WARNINGS AND PRECAUTIONS-

Hypersensitivity Reactions Including Anaphylaxis: Anaphylaxis has
occurred in 3.9% of treated patients. Administer KALBITOR in a
setting equipped to manage anaphylaxis and hereditary angioedema.
Given the similarity in hypersensitivity symptoms and acute HAE
symptoms, monitor patients closely for hypersensitivity reactions (5).

### ----ADVERSE REACTIONS-

 The most common adverse reactions occurring in ≥3% of KALBITORtreated patients and greater than placebo are headache, nausea, diarrhea, pyrexia, injection site reactions, and nasopharyngitis. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Dyax Corp. at 1-888-452-5248 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide

Revised: 12/2009

# FULL PRESCRIBING INFORMATION: CONTENTS\* WARNING: ANAPHYLAXIS

- 1 INDICATIONS AND USAGE
- 2 DOSAGE AND ADMINISTRATION
  - 2.1 Recommended Dosing
  - 2.2 Administration Instructions
- 3 DOSAGE FORMS AND STRENGTHS
- 4 CONTRAINDICATIONS
- 5 WARNINGS AND PRECAUTIONS
  - 5.1 Hypersensitivity Reactions, Including Anaphylaxis
- 6 ADVERSE REACTIONS
  - 6.1 Clinical Trials Experience
  - 6.2 Immunogenicity
- 7 DRUG INTERACTIONS
- 8 USE IN SPECIFIC POPULATIONS
  - 8.1 Pregnancy
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- 10 OVERDOSAGE
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- 12 CLINICAL PHARMACOLOGY
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  - 12.2 Pharmacodynamics
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- 13 NONCLINICAL TOXICOLOGY
  - 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
  - 13.2 Animal Toxicology
- 14 CLINICAL STUDIES
- 16 HOW SUPPLIED/STORAGE AND HANDLING
- 17 PATIENT COUNSELING INFORMATION

<sup>\*</sup>Sections or subsections omitted from the full prescribing information are not listed.

### **FULL PRESCRIBING INFORMATION**

### WARNING: ANAPHYLAXIS

Anaphylaxis has been reported after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR. [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)]

### 1 INDICATIONS AND USAGE

KALBITOR® (ecallantide) is indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older.

### 2 DOSAGE AND ADMINISTRATION

### 2.1 Recommended Dosing

The recommended dose of KALBITOR is 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If the attack persists, an additional dose of 30 mg may be administered within a 24 hour period.

### 2.2 Administration Instructions

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema.

KALBITOR should be refrigerated and protected from the light. KALBITOR is a clear, colorless liquid; visually inspect each vial for particulate matter and discoloration prior to administration. If there is particulate matter or discoloration, the vial should not be used.

Using aseptic technique, withdraw 1 mL (10 mg) of KALBITOR from the vial using a large bore needle. Change the needle on the syringe to a needle suitable for subcutaneous injection. The recommended needle size is 27 gauge. Inject KALBITOR into the skin of the abdomen, thigh, or upper arm. Repeat the procedure for each of the 3 vials comprising the KALBITOR dose. The injection site for each of the injections may be in the same or in different anatomic locations (abdomen, thigh, upper arm). There is no need for site rotation. Injection sites should be separated by at least 2 inches (5 cm) and away from the anatomical site of attack.

The same instructions apply to an additional dose administered within 24 hours. Different injection sites or the same anatomical location (as used for the first administration) may be used.

### 3 DOSAGE FORMS AND STRENGTHS

KALBITOR is a clear, colorless liquid free of preservatives. Each vial of KALBITOR contains ecallantide at a concentration of 10 mg/mL.

### 4 CONTRAINDICATIONS

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. [see Warnings and Precautions (5.1)].

# 5 WARNINGS AND PRECAUTIONS

# 5.1 Hypersensitivity Reactions, Including Anaphylaxis

Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR. In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical studies, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruritus, rhinorrhea, sneezing, nasal congestion, throat irritation, urticaria, wheezing, and hypotension. These reactions occurred within the first hour after dosing.

Other adverse reactions indicative of hypersensitivity reactions included the following: pruritus (5.1%), rash (3.1%), and urticaria (2.0%).

Patients should be observed for an appropriate period of time after administration of KALBITOR, taking into account the time to onset of anaphylaxis seen in clinical trials. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

KALBITOR should not be administered to any patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4)].

### 6 ADVERSE REACTIONS

Hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR [see Contraindications (4) and Warnings and Precautions (5.1)].

# 6.1 Clinical Trials Experience

Because clinical trials are conducted under varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety data described below reflect exposure to KALBITOR in 255 patients with HAE treated with either intravenous or subcutaneous KALBITOR. Of the 255 patients,

66% of patients were female and 86% were Caucasian. Patients treated with KALBITOR were between the ages of 10 and 78 years.

Overall, the most common adverse reactions in 255 patients with HAE were headache (16.1%), nausea (12.9%), fatigue (11.8%), diarrhea (10.6%), upper respiratory tract infection (8.2%), injection site reactions (7.4%), nasopharyngitis (5.9%), vomiting (5.5%), pruritus (5.1%), upper abdominal pain (5.1%), and pyrexia (4.7%). Anaphylaxis was reported in 3.9% of patients with HAE. Injection site reactions were characterized by local pruritus, erythema, pain, irritation, urticaria, and/or bruising.

The incidence of adverse reactions below is based upon 2 placebo-controlled, clinical trials (EDEMA3® and EDEMA4®) in a total of 143 unique patients with HAE. Patients were treated with KALBITOR 30 mg subcutaneous or placebo. Patients were permitted to participate sequentially in both placebo-controlled trials; safety data collected during exposure to KALBITOR was attributed to treatment with KALBITOR, and safety data collected during exposure to placebo was attributed to treatment with placebo. Table 1 shows adverse reactions occurring in ≥3% of KALBITOR-treated patients that also occurred at a higher rate than in the placebo-treated patients in the two controlled trials (EDEMA3 and EDEMA4) of the 30 mg subcutaneous dose.

Table 1: Adverse Reactions Occurring at ≥3% and Higher than Placebo in 2 Placebo Controlled Clinical Trials in Patients with HAE Treated with KALBITOR

	Chilical Thais in Fatients with HAE Treated With KALBITOR	
	KALBITOR	Placebo
	N=100	N=81
Adverse Reactions	n (%) <sup>8</sup>	n (%) <sup>s</sup>
Headache	8 (8%)	6 (7%)
Nausea	5 (5%)	1 (1%)
Diarrhea	4 (4%)	3 (4%)
Pyrexia	4 (4%)	0
Injection site reactions	3 (3%)	1 (1%)
Nasopharyngitis	3 (3%)	0

Patients experiencing more than 1 event with the same preferred term are counted only once for that preferred term.

Some patients in EDEMA3 and EDEMA4 received a second, open-label 30 mg subcutaneous dose of KALBITOR within 24 hours following the initial dose. Adverse reactions reported by these patients who received the additional 30 mg subcutaneous dose of KALBITOR were consistent with those reported in the patients receiving a single dose.

# 6.2 Immunogenicity

In the KALBITOR HAE program, patients developed antibodies to KALBITOR. Rates of seroconversion increased with exposure to KALBITOR over time. Overall, 7.4% of patients seroconverted to anti-ecallantide antibodies. Neutralizing antibodies to ecallantide were determined *in vitro* to be present in 4.7% of patients.

Anti-ecallantide and anti-P. pastoris IgE antibodies were also detected. Patients who seroconvert may be at a higher risk of a hypersensitivity reaction. The long-term effects of antibodies to KALBITOR are not known.

The test results for the ecallantide program were determined using one of two assay formats: ELISA and bridging electrochemiluminescence (ECL). As with all therapeutic proteins, there is a potential for immunogenicity with the use of KALBITOR. The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to KALBITOR with the incidence of antibodies to other products may be misleading.

# 7 DRUG INTERACTIONS

No formal drug interactions studies were performed. No *in vitro* metabolism studies were performed.

# 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled trials of KALBITOR in pregnant women. KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Because animal reproductive studies are not always predictive of human response, KALBITOR should be used during pregnancy only if clearly needed.

In rats, intravenous KALBITOR at an intravenous dose approximately 13 times the maximum recommended human dose (MRHD) on a mg/kg basis caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. No development toxicity was observed in rats that received an intravenous dose approximately 8 times the MRHD on a mg/kg basis. There were no adverse effects of KALBITOR on embryofetal development in rats that received subcutaneous doses up to approximately 2.4 times the MRHD on an AUC basis, and in rabbits that received intravenous doses up to approximately 6 times the MRHD on an AUC basis.

# 8.2 Labor and Delivery

No information is available on the effects of KALBITOR during labor and delivery.

# 8.3 Nursing Mothers

It is not known whether ecallantide is excreted in human milk. Caution should be exercised when ecallantide is administered to a nursing woman.

### 8.4 Pediatric Use

Safety and effectiveness of KALBITOR in patients below 16 years of age have not been established.

### 8.5 Geriatric Use

Clinical trials of KALBITOR did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

### 10 OVERDOSAGE

There have been no reports of overdose with KALBITOR. HAE patients have received single doses up to 90 mg intravenously without evidence of dose-related toxicity. No deaths occurred in monkeys that received intravenous or subcutaneous doses up to 25 mg/kg (approximately 22 times the MRHD on an AUC basis).

### 11 DESCRIPTION

KALBITOR (ecallantide) is a human plasma kallikrein inhibitor for injection for subcutaneous use.

KALBITOR is a clear and colorless, sterile, and nonpyrogenic solution. Each vial contains 10 mg ecallantide as the active ingredient, and the following inactive ingredients: 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, and 8 mg sodium chloride in water for injection, USP. KALBITOR is preservative free, with a pH of approximately 7.0. A 30 mg dose is supplied as 3 vials each containing 1 mL of 10 mg/mL KALBITOR. Each vial contains a slight overfill. Vials are intended for single use. Ecallantide is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology.

### 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on Chromosome 11q and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kininogen to bradykinin by the protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During

attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Bradykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein. KALBITOR binds to plasma kallikrein and blocks its binding site, inhibiting the conversion of HMW kininogen to bradykinin. By directly inhibiting plasma kallikrein, KALBITOR reduces the conversion of HMW kininogen to bradykinin and thereby treats symptoms of the disease during acute episodic attacks of HAE.

### 12.2 Pharmacodynamics

No exposure-response relationships for KALBITOR to components of the complement or kallikrein-kinin pathways have been established.

The effect of KALBITOR on activated partial thromboplastin time (aPTT) was measured because of potential effect on the intrinsic coagulation pathway. Prolongation of aPTT has been observed following intravenous dosing of KALBITOR at doses ≥20 mg/m². At 80 mg administered intravenously in healthy subjects, aPTT values were prolonged approximately two-fold over baseline values and returned to normal by 4 hours post-dose.

For patients taking KALBITOR, no significant QT prolongation has been seen. In a randomized, placebo-controlled trial (EDEMA4) studying the 30 mg subcutaneous dose versus placebo, 12-lead ECGs were obtained at baseline, 2 hours and 4 hours post-dose (covering the time of expected  $C_{max}$ ), and at follow-up (day 7). ECGs were evaluated for PR interval, QRS complex, and QTc interval. KALBITOR had no significant effect on the QTc interval, heart rate, or any other components of the ECG.

### 12.3 Pharmacokinetics

Following the administration of a single 30 mg subcutaneous dose of KALBITOR to healthy subjects, a mean ( $\pm$  standard deviation) maximum plasma concentration of 586  $\pm$  106 ng/mL was observed approximately 2 to 3 hours post-dose. The mean area under the concentration-time curve was  $3017 \pm 402$  ng\*hr/mL. Following administration, plasma concentration declined with a mean elimination half-life of  $2.0 \pm 0.5$  hours. Plasma clearance was  $153 \pm 20$  mL/min and the volume of distribution was  $26.4 \pm 7.8$  L. Based on a population pharmacokinetic analysis, body weight, age, and gender were not found to affect KALBITOR exposure significantly. Ecallantide is a small protein (7054 Da) and renal elimination in the urine of treated subjects has been demonstrated.

No pharmacokinetic data are available in patients or subjects with hepatic or renal impairment.

### 13 NONCLINICAL TOXICOLOGY

# 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There are no animal or human studies to assess the carcinogenic or mutagenic potential of KALBITOR (ecallantide).

KALBITOR had no effects on fertility and reproductive performance in rats at subcutaneous doses up to 25 mg/kg/day (approximately 21 times the MRHD on a mg/kg basis).

## 13.2 Animal Toxicology

Reproductive Toxicology Studies

KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Treatment of rats with an intravenous dose of 15 mg/kg/day (approximately 13 times the MRHD on a mg/kg basis) caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. However, no development toxicity was observed in rats that received an intravenous dose of 10 mg/kg/day (approximately 8 times the MRHD on a mg/kg basis). KALBITOR was not teratogenic in rats at subcutaneous doses up to 20 mg/kg/day (approximately 2.4 times the MRHD on an AUC basis) and rabbits that received intravenous doses up to 5 mg/kg/day (approximately 6 times the MRHD on an AUC basis).

### 14 CLINICAL STUDIES

The safety and efficacy of KALBITOR was evaluated in 2 randomized, double-blind, placebo-controlled trials (EDEMA4 and EDEMA3) in 168 patients with HAE. Patients having an attack of hereditary angioedema, at any anatomic location, with at least 1 moderate or severe symptom, were treated with 30 mg subcutaneous KALBITOR or placebo. Because patients could participate in both trials, a total of 143 unique patients participated. Of the 143 patients, 94 were female, 123 were Caucasian, and the mean age was 36 years. There were 64 patients with abdominal attacks, 55 with peripheral attacks, and 24 with laryngeal attacks.

In both trials, the effects of KALBITOR were evaluated using the Mean Symptom Complex Severity (MSCS) score and the Treatment Outcome Score (TOS). These measures evaluated the severity of attack symptoms at all anatomical locations (MSCS score) and response to therapy (TOS).

MSCS score is a point-in-time measure of symptom severity. At baseline, 4 hours, and 24 hours, patients rated the severity on a categorical scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for symptoms at each affected anatomical location. Ratings were averaged to obtain the MSCS score. A decrease in MSCS score reflected an improvement in symptoms.

TOS is a measure of symptom response to treatment. At 4 hours and 24 hours, patient assessment of response characterized by their change from baseline in symptom severity and collected by anatomic site of attack involvement, was recorded on a categorical scale (significant improvement [100], improvement [50], same [0], worsening [-50], significant worsening [-100]). The response at each anatomic site was weighted by baseline severity and then the weighted scores across all involved sites were averaged to calculate the TOS. A TOS value >0 reflected an improvement in symptoms from baseline.

### **EDEMA4**

EDEMA4 was a randomized, double-blind, placebo-controlled trial in which 96 patients were randomized 1:1 to receive KALBITOR 30 mg subcutaneous or placebo for acute attacks of HAE. The primary endpoint was the change from baseline in MSCS score at 4 hours, and the TOS at 4 hours was a key secondary endpoint. Patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients with placebo and the results were statistically significant (Table 2). At 24 hours, patients treated with KALBITOR also demonstrated a greater decrease from baseline in the MSCS than placebo (-1.5 vs. -1.1; p = 0.04) and a greater TOS (89 vs. 55, p = 0.03).

Table 2: Change in MSCS Score and TOS at 4 Hours

	EDE	EDEMA4		MA3
	KALBITOR (N=48)	Placebo (N=48)	KALBITOR (N=36)	Placebo (N=36)
Change in MS	CS Score at 4 Hours			
n	47	42	34	35
Mean	-0.8	-0.4	-1.1	-0.6
95% CI	-1.0, -0.6	-0.6, -0.1	-1.4, -0.8,	-0.8, -0.4
P-value	0.0	10	0.0	•
TOS at 4 Hour	<u>s</u>			
n	47	42	34	35
Mean	53	8	63	36
95% CI	39, 68	-12, 28	49, 76	17, 54
P-value	0.00	)3	0.04	

MSCS: Mean Symptom Complex Severity

TOS: Treatment Outcome Score

CI: confidence interval

More patients in the placebo group (24/48, 50%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (16/48, 33%).

Some patients reported improvement following a second 30 mg subcutaneous dose of KALBITOR, administered within 24 hours following the initial dose for symptom persistence or relapse, but efficacy was not systematically assessed for the second dose.

### EDEMA3

EDEMA3 was a randomized, double-blind, placebo-controlled trial in which 72 patients were randomized 1:1 to receive KALBITOR or placebo for acute attacks of HAE. EDEMA3 was similar in design to EDEMA4 with the exception of the order of the prespecified efficacy endpoints. In EDEMA3, the primary endpoint was the TOS at 4 hours, and the key secondary efficacy endpoint was the change from baseline in MSCS at 4 hours. As in EDEMA4, patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients treated with placebo and the results were statistically significant (Table 2).

In addition, more patients in the placebo group (13/36, 36%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (5/36, 14%).

## 16 HOW SUPPLIED/STORAGE AND HANDLING

KALBITOR (ecallantide) is supplied as three 10 mg/mL single-use vials packaged in a carton. Each vial contains 10 mg of ecallantide. Each vial contains a slight overfill.

• NDC (47783-101-01): 3 single-use vials in 1 carton

KALBITOR should be kept refrigerated (2°C to 8°C/36°F to 46°F). Vials removed from refrigeration should be stored below 86°F/30°C and used within 14 days or returned to refrigeration until use.

Protect vials from light until use.

Do not use beyond the expiration date.

### 17 PATIENT COUNSELING INFORMATION

See Medication Guide

- Patients should be advised that KALBITOR may cause anaphylaxis and other hypersensitivity reactions. Patients should be advised that KALBITOR should be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Patients who have known clinical hypersensitivity to KALBITOR should be instructed not to receive additional doses of KALBITOR. [see Boxed Warning, Contraindications (4), and Warnings and Precautions (5.1)]
- Patients should be advised to consult the Medication Guide for additional information regarding the risk of anaphylaxis and other hypersensitivity reactions.

### **Medication Guide**

# KALBITOR® (KAL-bi-tor)

### (ecallantide)

Read this Medication Guide before you start receiving KALBITOR and before each treatment. There may be new information. This Medication Guide does not take the place of talking to your doctor about your medical condition or your treatment.

# What is the most important information that I should know about KALBITOR?

Serious allergic reactions may happen in some people who receive KALBITOR. These allergic reactions can be life-threatening and usually happen within 1 hour after receiving KALBITOR.

- KALBITOR should be given to you by a doctor or nurse in a healthcare setting where serious allergic reactions and hereditary angioedema (HAE) can be treated.
- Symptoms of a serious allergic reaction to KALBITOR can be similar to the symptoms of HAE, the condition that you are being treated for. Your doctor or nurse should watch you for any signs of a serious allergic reaction after treatment with KALBITOR.
- Tell your doctor or nurse right away if you have any of these symptoms of a serious allergic reaction during or after treatment with KALBITOR:
  - wheezing, shortness of breath, cough, chest tightness, or trouble breathing
  - dizziness, fainting, fast or weak heartbeat, or feeling nervous
  - reddening of the face, itching, hives, or feeling warm
  - swelling of the throat or tongue, throat tightness, hoarse voice, or trouble swallowing
  - runny nose or sneezing

### What is KALBITOR?

KALBITOR is a prescription medicine used to treat sudden attacks of hereditary angioedema (HAE).

KALBITOR is not a cure for HAE.

It is not known if KALBITOR is safe and effective in children under 16 years of age.

### Who should not receive KALBITOR?

Do not receive KALBITOR if you are allergic to KALBITOR.

### What should I tell my doctor before I receive KALBITOR?

Before receiving KALBITOR, tell your doctor if you:

- have ever had an allergic reaction to KALBITOR. See "Who should not take KALBITOR?"
- are pregnant or plan to become pregnant. It is not known if KALBITOR will harm your unborn baby.
- are breast-feeding or plan to breast-feed. It is not known if KALBITOR passes into your breast milk.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list of them to show to your doctor and pharmacist when you get a new medicine.

### How will I receive KALBITOR?

For each dose, you will receive 3 injections just under the skin (subcutaneous or SC injections) of your abdomen, thigh, or upper arm.

### What are the possible side effects?

KALBITOR can cause serious allergic reactions. See "What is the most important information I should know about KALBITOR?").

Common side effects of KALBITOR include:

- headache
- nausea
- diarrhea
- fever
- · injection site reactions, such as redness, rash, swelling, itching, or bruising
- stuffy nose

Call your doctor for advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

### General information about KALBITOR

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. This Medication Guide gives you the most important information about KALBITOR. If you would like more information, talk with your doctor. You can ask your pharmacist or doctor for information about KALBITOR that is written for health professionals.

### What are the ingredients of KALBITOR?

Active Ingredient: ecallantide

Inactive ingredients: disodium hydrogen orthophosphate (dihydrate), monopotassium phosphate, potassium chloride, sodium chloride in water for injection.

Manufactured for: Dyax Corp.

300 Technology Square, Cambridge, MA 02139

Issued December 2009

This Medication Guide has been approved by the U.S. Food and Drug Administration.

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In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

Attachment C

U.S. Patent No. 7,276,480

# **ATTACHMENT C**



### US007276480B1

# (12) United States Patent Ladner et al.

(10) Patent No.:

US 7,276,480 B1

(45) Date of Patent:

\*Oct. 2, 2007

# (54) PREVENTION AND REDUCTION OF BLOOD LOSS

(75) Inventors: Robert C. Ladner, Ijamsville, MD

(US); Arthur C. Ley, Newton, MA (US); Shirish Hirani, Arlington, MA (US); Anthony Williams, Melrose, MA

(US)

(73) Assignee: Dyax Corp., Cambridge, MA (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-

claimer.

(21) Appl. No.: 11/323,261

(22) Filed: Dec. 30, 2005

(51) Int. Cl.

A61K 38/16 (2006.01) C07K 14/00 (2006.01)

(52) U.S. Cl. ..... 514/12; 530/324

(58) Field of Classification Search ...... 514/12;

See application file for complete search history.

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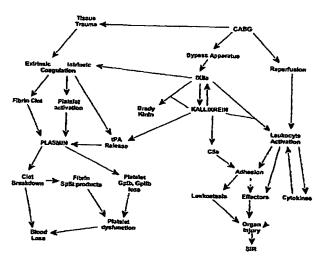
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(74) Attorney, Agent, or Firm—Fish & Richardson P.C.

### (57) ABSTRACT

Methods are described for preventing or reducing ischemia and/or systemic inflammatory response in a patient such as perioperative blood loss and/or systemic inflammatory response in a patient subjected to cardiothoracic surgery, e.g. coronary artery bypass grafting and other surgical procedures, especially when such procedures involve extra-corporeal circulation, such as cardiopulmonary bypass.

### 4 Claims, 4 Drawing Sheets



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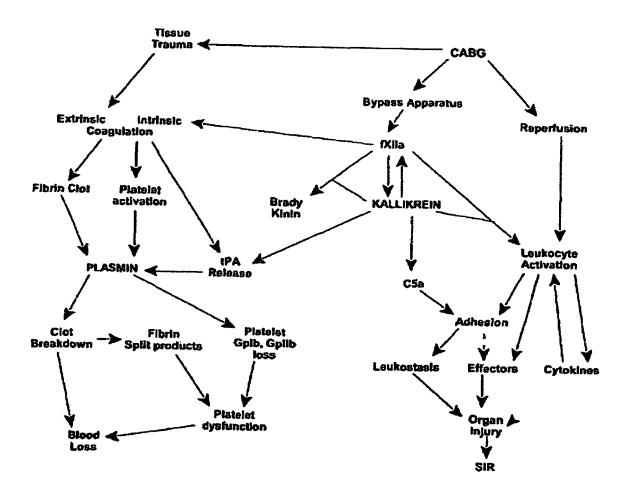
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Figure 1



# Figure 2

SAOX1 CG ACT TIT AAC GAC AAC TTG AGA AGA TCA AAA AAC AAC TAA TTA TTC GAA **ACG** ATG AGA TTC CCA TCT ATC TTC ACT GCT GTT TTG TTC GCT GCT S F P I F T Αν TCC TCT GCT TTG GCT GCT CCA GTT AAC ACC ACT ACT GAA GAC GAG ACT L A A P V N T T T E GCT CAA ATT CCT GCT GAG GCT GTC ATC GGT TAC TCT GAC TTG GAA GGT EAVIGY S GAC TTC GAC GTC GCT GTT TTG CCA TTC TCT AAC TCT ACT AAC AAC GGT F S N S L P A T TTG TTG TTC ATC AAC ACT ACC ATC GCT TCT ATC GCT GCT-AAG GAG GAA I N T T I A S I A A GET GTT TCC CTC GAG AAG AGA GAG GCT ATG CAC TCT TTC TGT GCT TTC M H aag get gac gat get oeg tge aga get get eac eea aga tgg tte tte R AAC ATC TTC ACG CGT CAA TGC GAG GAG TTC ATC TAC GGT GGT TGT GAG F R Q C E F I GGT AAC CAA AAC AGA TTC GAG TCT CTA GAG GAG TGT AAG AAG ATG TGT E S L E E K Q N R E G N EcoR I ACT AGA GAC TAG TAA GAA TTC GCC TTA GAC ATG ACT GTT CCT CAG TTC D | \* \* T R 3'AOX1

AAG TTG GGC ACT TAC GAG AAG 3'AOXI

#### FIGURE 3A

```
(amino acids 3-60) ----MHSFCAFKA-DDGPCRAAHPRWFFNIFTRQCEEFIYGG
 SEQ ID 2:
 SEQ ID 4:
                                ----MHSFCAPKA-DDGPCKANHLRFFFNIFTRQCEEFSYGG
 SEQ ID 5:
                                ---- MHSFCAPKA-DDGHCKANHQRFFFNIFTRQCEEFTYGG
 SEQ ID 6:
                                ----MHSFCAPKA-DDGHCKANHQRFFFNIFTRQCEQFTYGG
 SEQ ID 7:
                                ----MHSFCAFKA-DDGHCKASLPRFFFN1FTRQCEEF1YGG
 SEQ ID 8:
                               ----mhsfcafka-ddghckanhqrfffniftrqceefsygg
 SEQ ID 9:
                               ----mhspcakfa-ddghckgahlrfffniftrqcebfiygg
 SEQ ID 10:
                               ----MHSPCAFKA-DDGRCKGAHLRFFFNIFTRQCBBFIYGG
 SEO ID 11:
                               ----mhsfcafka-dggrcrgahprwffniftrqceefsygg
 SEQ ID 12:
                               ----mhspcafka-ddgpcraahprwffniftrqceefsygg
 SEQ ID 13:
                               ---- MHSFCAFKA-DVGRCRGAHPRWFFNIFTRQCEEFSYGG
 SEQ ID 14:
                               ---- MHSFCAFKA-DVGRCRGAQPRFFFNIFTRQCEEFSYGG
 SEQ ID 15:
                               ----mhsfcafka-ddgscraahlrwffniftrqceefsygg
 SEQ ID 16:
                               ----mhsfcafka-eggscraahqrwffniftrqceefsygg
 SEQ ID 17:
                               ----mhsfcafka-ddgpcrgahlrfffniftrqceefsygg
 SEQ ID 18:
                               ----MHSFCAFKA-DDGHCRGALPRWFFNIFTRQCEEFSYGG
SEQ ID 19:
                               ----mhspcafka-dsgncrgnlprfffniftrocbefsygg
SEQ ID 20:
                               ----mhsfcafka-dsgrcrgnhqrfffniftrqceefsygg
SEQ ID 21:
                               ----MHSFCAFKA-DGGRCRAIQPRWFFNIFTRQCEEFSYGG
SEQ ID 22:
                             ----mhsfcafka-ddgrcrgahprwffniftrqceepsygg
                            ----RPDFCLEPP-YTGPCKARIIRYFYNAKAGLCQTPVYGG
----KEDSCQLGY-SAGPCMGMTSRYFYNGTSMACETFQYGG
BPTI (SEQ ID 29):
ITI-D1 (SEQ ID 30):
ITI-D2 (SEQ ID 31):
                              ----TVAACNLPI-VRGPCRAFIQLWAFDAVKGKCVLFPYGG
LACI-D1 (SEQ ID 32):
                             ----mhsfcafka-ddgpckaimkrfffniftrqceefiygg
LACI-D2 (SEQ ID 33):
                             ----KPDFCFLEE-DPGICRGYITRYFYNNQTKQCERFKYGG
LACI-D3 (SEQ ID 34):
                             ----GPSWCLTPA-DRGLCRANENRFYYNSVIGKCRPFKYSG
HKI B9 (SEQ ID 35):
                             ----LPNVCAFPM-EKGPCQTYMTRWPFNFETGECELFAYGG
Ca3 (SEQ ID 36):
                              ----ETDICKLPK-DEGTCRDFILKWYYDPNTKSCARFWYGG
TFPI-2 D1 (SEQ ID 37):
                              ----NAEICLLPL-DYGPCRALLLRYYYDRYTQSCRQFLYGG
TFPI-2 D2 (SEQ ID 38):
                              ----VPKVCRLQVSVDDQCEGSTEKYFFNLSSMTCEKFPSGG
TFPI-2 D3 (SEQ ID 39):
                              ---- IPSFCYSPK-DEGLCSANVTRYYFNPRYRTCDAFTYTG
APP-I (SEQ ID 40):
                              ---RNREVCSEQA-ETGPCRAMISRWYFDVTEGKCAPFFYGG
EpiNE7 (SEQ ID 41):
                              ----RPDFCLEPP-YTGPCVAMPPRYFYNAKAGLCQTFVYGG
BITI-E7-141 (SEQ ID 42):
                              ----RPDFCQLGY-SAGPCVAMFPRYFYNGTSMACQTFVYGG
MUTT26A (SEQ ID 43):
                              ----RPDFCQLGY-SAGPCVAMPPRYFYNGASMACQTFVYGG
MUTQE (SEQ ID 44):
                              ---- RPDFCQLGY-SAGPCVAMFPRYFYNGTSMACETFVYGG
MUT1619 (SEQ ID 45):
                              ----RPDFCQLGY-SAGPCVGMFSRYFYNGTSMACQTFVYGG
EPI-HNE-1 (SEQ ID 46):
                              EAEARPDFCLEPP-YTGPCIAFFPRYFYNAKAGLCQTFVYGG
EPI-HNE-2 (SEQ ID 47):
                              -----AACNLPI-VRGPCIAFFPRWAFDAVKGKCVLFPYGG
EPI-HNE-3 (SEQ ID 48):
                              -----AACNLPI-VRGPCIAFFPRWAFDAVKGKCVLFPYGG
EPI-HNE-4 (SEQ ID 49):
                            -----EACNLPI-VRGPCIAFFPRWAFDAVKGKCVLFPYGG
DPI14 KR (SEQ ID 50):
                            -- Eavrevcseqa-etgpc1affprwyfdvtegkcapfpygg
DPI24 KR (SEQ ID 51):
                           --Eanaeiclipl-dygpciaffpryyydrytoscroflygg
--Eakpdfcflee-dpgicigffpryfynnoakocerfvygg
DPI68 KR (SEQ ID 52):
DPI84 KR (SEQ ID 53):
                             -- EAETDICKLPK-DEGTCIAFFPRWYYDPNTKSCARFVYGG
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Oct. 2, 2007

# FIGURE 3B

SEQ ID 2: (cont.)		Cegnonrfesleeckkmctrd
SEQ ID 4: (cont.)		CGGNQNRPESLEECKKMCTRD
SEQ ID 5: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 6: (cont.)		Cagnonrfesleeckkmctrd
SEQ ID 7: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 8: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 9: (cont.)		CEGNQNRFESLEECKKMCTRD
SEQ ID 10: (cont.)		CEGNQNRFESLEECKKMCTRD
SEQ ID 11: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 12: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 13: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 14: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 15: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 16: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 17: (cont.)		CGGNQNRFESLEBCKKMCTRD
SEQ ID 18: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 19: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 20: (cont.)		CGGNQNRFESLEECKKMCTRD
SEQ ID 21: (cont.)	•	CGGNQNRFESLEECKKMCTRD
SEQ ID 22: (cont.)		CGGNQNRFESLEECKKMCTRD
BPTI (SEQ ID 29):	(cont.)	CRAKRNNFKSAEDCMRTCGGA
ITI-D1 (SEQ ID 30):	(cont.)	CMGNGNNFVTEKECLQTCRTV
ITI-D2 (SEQ ID 31):	(cont.)	CQGNGNKFYSEKBCREYCGVP
LACI-D1 (SEQ ID 32):	(cont.)	CEGNQNRFESLEECKKMCTRD
LACI-D2 (SEQ ID 33):	(cont.)	CLGNMNNFETLEECKNICEDG
LACI-D3 (SEQ ID 34):	(cont.)	CGGNE NNFTSKQECLRACKKG
HKI B9 (SEQ ID 35):	(cont.)	CGGNSNNFLRKEKCEKFCKFT
C∝3 (SEQ ID 36):	(cont.)	CGGNENKFGSQKECEKVCAPV
TFPI-2 D1 (SEQ ID 37):	(cont.)	CEGNANNFYTWEACDDACWRI
TFPI-2 D2 (SEQ ID 38):	(cont.)	CHRNRIENRFPDEATCMGFCAPK
TFPI-2 D3 (SEQ ID 39):	(cont.)	CGGNDNNFVSREDCKRACAKA
APP-1 (SEQ ID 40):		CGGNRNNFDTEBYCMAVCGSA
EpiNE7 (SEQ ID 41):	(cont.)	CMGNGNNFKSAEDCMRTCGGA
BITI-E7-141 (SEQ ID 42)	:(cont.)	CMGNGNNFVTEKDCLQTCRGA
MUTT26A (SEQ ID 43):	(cont.)	CMGNGNNFVTEKDCLQTCRGA
MUTQE (SEQ ID 44):	(cont.)	CMGNGNNFVTEKDCLQTCRGA
MUT1619 (SEQ ID 45):	(cont.)	CMGNGNNFVTEKDCLQTCRGA
EPI-HNE-1 (SEQ ID 46):	(cont.)	CMGNGNNPKSAEDCMRTCGGA
EPI-HNE-2 (SEQ ID 47):	(cont.)	CQGNGNKPYSEKECREYCGVP
EPI-HNE-3 (SEQ ID 48):	(cont.)	CQGNGNKFYSEKECREYCGVP
EPI-HNE-4 (SEQ ID 49):	(cont.)	CQGNGNKFYSEKECREYCGVP
DPI14 KR (SEQ ID 50):	(cont.)	CGGNRNNFDTEEYCMAVCGSA
DPI24 KR (SEQ ID 51):	(cont.)	Cegnannfytweacddacwri
DP168 KR (SEQ ID 52):	(cont.)	CLGNMNNFETLEECKNICEDG
DP184 KR (SEQ ID 53):	(cont.)	CGGNENKFGSQKECEKVCAPV

### PREVENTION AND REDUCTION OF BLOOD LOSS

### RELATED APPLICATION

This application claims the benefit of U.S. application Ser. No. 10/456,986, filed Jun. 6, 2003, now U.S. Pat. No. 7,064,107, which claims the benefit from U.S. Provisional Application No. 60/387,239, filed Jun. 7, 2002, and U.S.

The entire teachings of the above applications are incorporated herein by reference.

### BACKGROUND OF THE INVENTION

Proteases are involved in a broad range of biological pathways. In particular, serine proteases such as kallikrein, plasmin, elastase, urokinase plasminogen activator, thrombin, human lipoprotein-associated coagulation inhibitor, and 20 coagulation factors such as factors VIIa, IXa, Xa, Xla, and XIIa have been implicated in pathways affecting blood flow. e.g., general and focal ischemia, tumor invasion, fibrinolysis, perioperative blood loss, and inflammation. Inhibitors of as potential drug targets for various ischemic maladies.

One such inhibitor, aprotinin (also called bovine pancreatic trypsin inhibitor or BPTI), obtained from bovine lung, has been approved in the United States for prophylactic use in reducing perioperative blood loss and the need for transfusion in patients undergoing cardiopulmonary bypass (CPB), e.g., in the course of a coronary artery bypass grafting procedure. Aprotinin is commercially available under the trade name TRASYLOL® (Bayer Corporation Pharmaceutical Division, West Haven, Conn.) and was 35 previously approved for use to treat pancreatitis. The effectiveness of aprotinin is associated with its relatively nonspecific abilities to inhibit a variety of serine proteases, including plasma kallikrein and plasmin. These proteases are important in a number of pathways of the contact 40 activation system (CAS).

CAS is initially activated when whole blood contacts the surface of foreign substrates (e.g., kaolin, glass, dextran sulfate, or damaged bone surfaces). Kallikrein, a serine protease, is a plasma enzyme that initiates the CAS cascade 45 leading to activation of neutrophils, plasmin, coagulation, and various kinins. Kallikrein is secreted as a zymogen (pre-kallikrein) that circulates as an inactive molecule until activated by a proteolytic event early in the contact activation cascade. Clearly, specific inhibition of kallikrein would 50 be a very attractive approach to control blood loss associated with CPB and the onset of systemic inflammatory response (SIR) as would be encountered during, for example, various invasive surgical procedures.

Despite being the only licensed compound for preventing 55 perioperative blood loss in CPB for coronary artery bypass grafting (CABG) procedures, aprotinin is not as widely used as would be expected. There are serious concerns regarding the use of this bovine polypeptide in patients who require CPB, and in particular the use of this compound in CABG 60 procedures. Aprotinin is not specific for kallikrein, but interacts with additional enzymes (e.g., plasmin) in multiple pathways. Thus, the mechanism of action of aprotinin is largely speculative, and the lack of precise understanding of what is affected during aprotinin treatment produces the risk 65 of complications during treatment. One frequently cited complication is uncontrolled thrombosis, due to aprotinin's

actions upon the fibrinolytic pathway. There is concern not only over such hyperacute events as major vessel thrombosis in the perioperative period, but also over graft patency after the CABG procedure. Furthermore, as a naturally occurring protein obtained from bovine lung, administration of aprotinin in humans can elicit severe hypersensitivity or anaphylactic or anaphylactoid reactions after the first and, more often, after repeat administration to patients. This is particularly of concern in the large number of patients who have Provisional Application No. 60/407,003, filed Aug. 28, 10 repeat CABG procedures. In addition, there is an increasing public concern regarding use of material derived from bovine sources as a potential vector for the transmission of bovine spongiform encephalopathy to humans.

These concerns make clear that a need remains for more 15 effective and more specific means and methods for preventing or reducing perioperative blood loss and the onset of SIR in a patient subjected to surgery resulting in activation of the CAS, such as CABG procedures in patients of CPB, or hip replacement.

### SUMMARY OF THE INVENTION

This invention is based on the discovery of peptides that inhibit serine proteases. Serine proteases such as, for specific serine proteases, therefore, have received attention 25 example, kallikrein, are involved in, for example, pathways leading to excessive perioperative blood loss and the onset of systemic inflammatory response. Preferred kallikrein peptide inhibitors include those described in U.S. Pat. Nos. 6,333,402 and 6,057,287 to Markland et al., the contents of which are incorporated herein by reference in their entirety. The invention is directed in part to the use of the peptides in therapeutic methods and compositions suitable for use in eliminating or reducing various ischemias, including but not limited to perioperative blood loss, and the onset of systemic inflammatory response. Perioperative blood loss results from invasive surgical procedures that lead to contact activation of complement components and the coagulation/ fibrinolysis systems. More specifically, the invention provides methods of using kallikrein inhibitors to reduce or prevent perioperative blood loss and a systemic inflammatory response in patients subjected to invasive surgical procedures, especially cardiothoracic surgeries.

In one embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide comprising the amino acid sequence: Xaal Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1), wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa56, Xaa57 or Xaa58 are each individually an amino acid or absent; Xaa10 is an amino acid selected from the group consisting of: Asp and Glu; Xaall is an amino acid selected from the group consisting of: Asp, Gly, Ser, Val, Asn, Ile, Ala and Thr, Xaa13 is an amino acid selected from the group consisting of: Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys and Gln; Xaa15 is an amino acid selected from the group consisting of: Arg, Lys, Ala, Ser, Gly, Met, Asn and Gln; Xaa16 is an amino acid selected from the group consisting of: Ala, Gly, Ser, Asp and Asn: Xaa17 is an amino acid selected from the group consisting of: Ala, Asn, Ser, Ile, Gly, Val, Gln and Thr, Xaa18 is an amino acid selected from the group consisting of: His, Leu, Gln and Ala; Xaa19 is an amino acid selected from the group

consisting of: Pro, Gln, Leu, Asn and Ile; Xaa21 is an amino acid selected from the group consisting of: Trp, Phe, Tyr, His and lle; Xaa22 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa23 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa31 is an 5 amino acid selected from the group consisting of: Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile and Thr; Xaa32 is an amino acid selected from the group consisting of: Glu, Gln, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly and Val; Xaa34 is an amino acid selected from the group consisting of: Thr, Ile, Ser, Val, 10 Ala, Asn, Gly and Leu; Xaa35 is an amino acid selected from the group consisting of: Tyr, Trp and Phe; Xaa39 is an amino acid selected from the group consisting of: Glu, Gly, Ala, Ser and Asp; Xaa40 is an amino acid selected from the group consisting of: Gly and Ala; Xaa43 is an amino acid 15 Xaa34 is Ile, Xaa35 is Tyr, Xaa39 is Glu. selected from the group consisting of: Asn and Gly; Xaa45 is an amino acid selected from the group consisting of: Phe and Tyr; and wherein the polypeptide inhibits kallikrein.

In a particular embodiment, the ischemia is perioperative blood loss due to a surgical procedure performed on the 20 patient. The surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In a particular embodiment, individual amino acid positions of SEQ ID NO:1 can be one or more of the following: 25 Xaa10 is Asp, Xaa11 is Asp, Xaa13 is Pro, Xaa15 is Arg, Xaa16 is Ala, Xaa17 is Ala, Xaa18 is His, Xaa19 is Pro, Xaa21 is Trp, Xaa31 is Glu, Xaa32 is Glu, Xaa34 is Ile, Xaa35 is Tyr, Xaa39 is Glu.

In another embodiment, the invention is directed to a 30 method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide comprising the amino acid sequence: Xaa1 Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 35 Xaa9 Xaa10 Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa5 Cys Xaa52 40 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1), wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa56, Xaa57 or Xaa58 are each individually an amino acid or absent; Xaa10 is an amino acid selected from the group consisting of: Asp and Glu; Xaall is an amino acid selected from the group consisting of: Asp, Gly, Ser, Val, Asn, Ile, Ala and Thr; Xaa13 is an amino acid selected from the group consisting of: Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys and Gin; Xaa15 is an amino acid selected from the group consisting of: Arg, Lys, Ala, Ser, Gly, Met, Asn and Gin; Xaa16 is an amino acid selected from the group consisting of: Ala, Gly, Ser, Asp and Asn; Xaa17 is an amino acid selected from the group consisting of: Ala, Asn, Ser, Ile, Gly, Val, Gin and Thr; Xaa18 is an amino acid selected from the group consisting of: His, Leu, Gin and Ala; Xaa19 is an amino acid selected 55 from the group consisting of: Pro, Gin, Leu, Asn and Ile; Xaa21 is an amino acid selected from the group consisting of: Trp, Phe, Tyr, His and Ile; Xaa22 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa23 is an amino acid selected from the group consisting of: Tyr 60 and Phe; Xaa31 is an amino acid selected from the group consisting of: Glu, Asp, Gin, Asn, Ser, Ala, Val, Leu, Ile and Thr, Xaa32 is an amino acid selected from the group consisting of: Glu, Gin, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly and Val; Xaa34 is an amino acid selected from the group 65 consisting of: Thr, Ile, Ser, Val, Ala, Asn, Gly and Leu; Xaa35 is an amino acid selected from the group consisting

of: Tyr, Trp and Phe; Xaa39 is an amino acid selected from the group consisting of: Glu, Gly, Ala, Ser and Asp; Xaa40 is an amino acid selected from the group consisting of: Gly and Ala; Xaa43 is an amino acid selected from the group consisting of: Asn and Gly; Xaa45 is an amino acid selected from the group consisting of: Phe and Tyr, and wherein the polypeptide inhibits kallikrein. In a particular embodiment, the surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting. In a particular embodiment, individual amino acid positions of SEQ ID NO:1 can be one or more of the following: Xaa10 is Asp, Xaa11 is Asp, Xaa13 is Pro, Xaa15 is Arg, Xaa16 is Ala, Xaa17 is Ala, Xaa18 is His, Xaa19 is Pro, Xaa21 is Trp, Xaa31 is Glu, Xaa32 is Glu,

In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In another embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In a particular embodiment, the ischemia can be perioperative blood loss due to a surgical procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In another embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In a particular embodiment,

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the ischemia can be perioperative blood loss due to a surgical procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a simplified diagram of major multiple pathways and related events involved in the contact activation system 10 and systemic inflammatory response (SIR) that can arise in a patient subjected to soft and bone tissue trauma such as that associated with a coronary artery bypass grafting (CABG) procedure, especially when the CABG procedure involves extra-corporeal blood circulation, such as cardiop- 15 ulmonary bypass (Bypass Apparatus). Arrows indicate activation from one component or event to another component or event in the cascade. Arrows in both directions indicate activating effects of components or events in both directions. Broken arrows indicate likely participation of one compo- 20 nent or event in the activation of another component or event. Abbreviations are as follows: "tPA"=tissue plasminogen activator, "C5a"=a protein component of the complement system; "fXIIa"=activator protein of prekallikrein to form active kallikrein; "Extrinsic"=extrinsic coagulation 25 system; "Intrinsic"=intrinsic coagulation system.

FIG. 2 shows a portion of a DNA and corresponding deduced amino acid for a KI polypeptide of the invention in plasmid pPIC-K503. The inserted DNA encodes the mat.alpha. prepro signal peptide of Saccharomyces cerevisiae 30 (underlined) fused in frame to the amino terminus of the PEP-1 KI polypeptide having the amino acid sequence enclosed by the boxed area. The amino acid sequence of the PEP-1 KI polypeptide shown in the boxed region is SEQ ID NO:2, and the corresponding nucleotide coding sequence of 35 the KI polypeptide is SEQ ID NO:3. The dashed arrows indicate the location and direction of two PCR primer sequences in AOX regions that were used to produce sequencing templates. DNA sequence for the entire nucleotide sequence of the figure comprises the structural coding 40 sequence for the fusion protein and is designated SEQ ID NO:27. The entire amino acid sequence is SEQ ID NO:28. The double underlined portion of the sequence indicates a diagnostic probe sequence. BstBI and EcoRI indicate locations of their respective palindromic, hexameric, restriction 45 procedures, to prevent or reduce perioperative blood loss endonuclease sites in the sequence. Asterisks denote translational stop codons.

FIGS. 3A and 3B show an alignment of amino acid sequences of the preferred embodiments of the invention, the native LACI sequence from which these variants were 50 derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOS:29-31 and 33-53). Cysteine residues are highlighted.

### DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention follows.

The invention is based on the discovery of a group of 60 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1) kallikrein inhibitor (KI) polypeptides that inhibit plasma kallikrein with a specificity that permits their use in improved methods of preventing or reducing ischemia such as, for example, perioperative blood loss and/or a systemic inflammatory response (SIR) induced by kallikrein, especially, for example, in patients undergoing surgical procedures and particularly surgical procedures involving cardio-

thoracic surgery, e.g., cardiopulmonary bypass (CPB), such as a coronary artery bypass graft (CABG) procedures. K's can be used specifically for, e.g., pediatric cardiac surgery, lung transplantation, total hip replacement and orthotopic liver transplantation, and to reduce or prevent perioperative stroke during CABG, extracorporeal membrane oxygenation (ECMO) and cerebrovascular accidents (CVA) during these procedures.

Cardiothoracic surgery is surgery of the chest area, most commonly the heart and lungs. Typical diseases treated by cardiothoracic surgery include coronary artery disease, tumors and cancers of the lung, esophagus and chest wall, heart vessel and valve abnormalities, and birth defects involving the chest or heart. Where cardiothoracic surgery is utilized for treatment, the risk of blood loss (e.g., surgeryinduced ischemia) and the onset of a systemic inflammatory response (SIR) is incurred. Surgery-induced SIR can result in severe organ dysfunction (systemic inflammatory response syndrome; SIRS).

Polypeptides Useful in the Invention

KI polypeptides useful in the invention comprise Kunitz domain polypeptides. In one embodiment these Kunitz domains are variant forms of the looped structure comprising Kunitz domain 1 of human lipoprotein-associated coagulation inhibitor (LACI) protein. LACI contains three internal, well-defined, peptide loop structures that are paradigm Kunitz domains (Girard, T. et al., 1989. Nature, 338:518-520). The three Kunitz domains of LACl confer the ability to bind and inhibit kallikrein, although not with exceptional affinity. Variants of Kunitz domain 1 of LACI described herein have been screened, isolated and bind kallikrein with enhanced affinity and specificity (see, for example, U.S. Pat. Nos. 5,795,865 and 6,057,287, incorporated herein by reference). An example of a preferred polypeptide useful in the invention has the amino acid sequence defined by amino acids 3-60 of SEQ ID NO:2.

Every polypeptide useful in the invention binds kallikrein, and preferred polypeptides are also kallikrein inhibitors (KI) as determined using kallikrein binding and inhibition assays known in the art. The enhanced affinity and specificity for kallikrein of the variant Kunitz domain polypeptides described herein provides the basis for their use in cardiothoracic surgery, e.g., CPB and especially CABG surgical and/or the onset of SIR in patients undergoing such procedures. The KI polypeptides used in the invention have or comprise the amino acid sequence of a variant Kunitz domain polypeptide originally isolated by screening phage display libraries for the ability to bind kallikrein.

KI polypeptides useful in the methods and compositions of the invention comprise a Kunitz domain polypeptide comprising the amino acid sequence:

Xaa1 Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 55 Xaall Gly Xaal3 Cys Xaal5 Xaal6 Xaal7 Xaal8 Xaal9 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53

"Xaa" refers to a position in a peptide chain that can be any of a number of different amino acids. For example, for the KI peptides described herein, Xaa10 can be Asp or Glu; Xaall can be Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr; Xaal3 can be Pro, Arg, His, Asn, Ser, Thr, Ala, Gly, Lys or Gln; Xaa15 can be Arg, Lys, Ala, Ser, Gly, Met, Asn or Gln; Xaa16 can be Ala, Gly, Ser, Asp or Asn; Xaa17 can be Ala,

Asn, Ser, Ile, Gly, Val, Gln or Thr; Xaa18 can be His, Leu, Gln or Ala; Xaa19 can be Pro, Gln, Leu, Asn or Ile; Xaa21 can be Trp, Phe, Tyr, His or Ile; Xaa31 can be Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile or Thr; Xaa32 can be Glu, Gln, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly or Val; Xaa34 can be 5 Ile, Thr, Ser, Val, Ala, Asn, Gly or Leu; Xaa35 can be Tyr, Trp or Phe; Xaa39 can be Glu, Gly, Ala, Ser or Asp. Amino acids Xaa6, Xaa7, Xaa8, Xaa9, Xaa20, Xaa24, Xaa25, Xaa26, Xaa27, Xaa28, Xaa29, Xaa41, Xaa42, Xaa44, Xaa46, Xaa47, Xaa48, Xaa49, Xaa50, Xaa52, Xaa53 and 10 Xaa54 can be any amino acid. Additionally, each of the first four and at last three amino acids of SEQ ID NO: 1 can optionally be present or absent and can be any amino acid,

Peptides defined according to SEQ ID NO:1 form a set of 15 polypeptides that bind to kallikrein. For example, in a preferred embodiment of the invention, a KI polypeptide useful in the methods and compositions of the invention has the following variable positions: Xaall can be Asp, Gly, Ser or Val; Xaa13 can be Pro, Arg, His or Asn; Xaa15 can be Arg 20 or Lys; Xaa16 can be Ala or Gly; Xaa17 can be Ala, Asn, Ser or Ile; Xaa18 can be His, Leu or Gln; Xaa19 can be Pro, Gln or Leu; Xaa21 can be Trp or Phe; Xaa31 is Glu; Xaa32 can be Glu or Gln; Xaa34 can be Ile, Thr or Ser; Xaa35 is Tyr; and Xaa39 can be Glu, Gly or Ala.

A more specific embodiment of the claimed invention is defined by the following amino acids at variable positions: Xaa10 is Asp; Xaa11 is Asp; Xaa13 can be Pro or Arg; Xaa15 is Arg; Xaa16 can be Ala or Gly; Xaa17 is Ala; Xaa18 is His; Xaa19 is Pro; Xaa21 is Trp; Xaa31 is Glu; Xaa32 is 30 Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Glu; Xaa34 can be Ile or Ser; Xaa35 is Tyr; and Xaa39 is Gly.

Also encompassed within the scope of the invention are peptides that comprise portions of the polypeptides binding domains for specific kallikrein epitopes. Such fragments of the polypeptides described herein would also be encompassed.

KI polypeptides useful in the methods and compositions described herein comprise a Kunitz domain. A subset of the 40 Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Asn sequences encompassed by SEQ ID NO:1 are described by the following (where not indicated, "Xaa" refers to the same set of amino acids that are allowed for SEQ ID NO:1):

Met His Ser Phe Cys Ala Phe Lys Ala Xaa10 Xaa11 Gly Phe Phe Asn Ile Phe Thr Arg Gln Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Gly Asn Gln Asn Arg Phe Ghu Ser Leu Ghu Ghu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:54).

Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2),

Cys Lys Ala Asn His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:4),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His 60 Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Thr Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:5),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His 65 Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Gln Phe Thr Tyr Gly Gly Cys Ala Gly Asn

Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:6),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala Ser Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:7),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:8).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Gly Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:9),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Lys Gly Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe lle Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:10).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:11),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:12),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr described herein. For example, polypeptides could comprise 35 Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:13),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly Ala Gln Pro Arg Phe Phe Phe Asn Ile Phe Thr Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO: 14),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Ser Cys Arg Ala Ala His Leu Arg Trp Phe Phe Asn Ile Phe Thr Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Arg Xaa21 45 Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:15),

Met His Ser Phe Cys Ala Phe Lys Ala Glu Gly Gly Ser Cys Arg Ala Ala His Gin Arg Trp Phe Phe Asn Ile Phe Thr Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro 50 Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:16),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Gly Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro 55 Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:17),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Arg Gly Ala Leu Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:18),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Asn Cys Arg Gly Asn Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:19),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Arg Cys Arg Gly Asn His Gin Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:20),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Ala Ile Gin Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:21),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:22).

FIGS. 3A and 3B provides an amino acid sequence alignment of these sequences, the native LACI sequence from which these variants were derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOS: 29-31 and 33-53).

The KI polypeptides useful in the methods and compositions described herein can be made synthetically using any standard polypeptide synthesis protocol and equipment. For example, the stepwise synthesis of a KI polypeptide described herein can be carried out by the removal of an 25 amino (N) terminal-protecting group from an initial (i.e., carboxy-terminal) amino acid, and coupling thereto of the carboxyl end of the next amino acid in the sequence of the polypeptide. This amino acid is also suitably protected. The carboxyl group of the incoming amino acid can be activated to react with the N-terminus of the bound amino acid by formation into a reactive group such as formation into a carbodiimide, a symmetric acid anhydride, or an "active ester" group such as hydroxybenzotriazole or pentafluorophenyl esters. Preferred solid-phase peptide synthesis 35 methods include the BOC method, which utilizes tertbutyloxycarbonyl as the .alpha.-amino protecting group, and the FMOC method, which utilizes 9-fluorenylmethloxycarbonyl to protect the .alpha.-amino of the amino acid residues. Both methods are well known to those of skill in the 40 art (Stewart, J. and Young, J., Solid-Phase Peptide Synthesis (W. H. Freeman Co., San Francisco 1989); Merrifield, J., 1963. Am. Chem. Soc., 85:2149-2154; Bodanszky, M. and Bodanszky, A., The Practice of Peptide Synthesis (Springer-Verlag, New York 1984), the entire teachings of these 45 references is incorporated herein by reference). If desired, additional amino- and/or carboxy-terminal amino acids can be designed into the amino acid sequence and added during polypeptide synthesis.

polypeptides useful in the compositions and methods of the invention can be produced by recombinant methods using any of a number of cells and corresponding expression vectors, including but not limited to bacterial expression vectors, yeast expression vectors, baculovirus expression 55 vectors, mammalian viral expression vectors, and the like. Kunitz domain polypeptides and KI polypeptides useful in the compositions and methods of the invention can also be produced transgenically using nucleic acid molecules comprising a coding sequence for a Kunitz domain or KI 60 polypeptide described herein, wherein the nucleic acid molecule can be integrated into and expressed from the genome of a host animal using transgenic methods available in the art. In some cases, it could be necessary or advantageous to fuse the coding sequence for a Kunitz domain polypeptide or 65 a KI polypeptide comprising the Kunitz domain to another coding sequence in an expression vector to form a fusion

polypeptide that is readily expressed in a host cell. Preferably, the host cell that expresses such a fusion polypeptide also processes the fusion polypeptide to yield a Kunitz domain or KI polypeptide useful in the invention that contains only the desired amino acid sequence. Obviously, if any other amino acid(s) remain attached to the expressed Kunitz domain or KI polypeptide, such additional amino acid(s) should not diminish the kallikrein binding and/or kallikrein inhibitory activity of the Kunitz domain or KI polypeptide so as to preclude use of the polypeptide in the methods or compositions of the invention.

A preferred recombinant expression system for producing KI polypeptides useful in the methods and compositions described herein is a yeast expression vector, which permits 15 a nucleic acid sequence encoding the amino acid sequence for a KI polypeptide or Kunitz domain polypeptide to be linked in the same reading frame with a nucleotide sequence encoding the mat.alpha. prepro leader peptide sequence of Saccharomyces cerevisiae, which in turn is under the control 20 of an operable yeast promoter. The resulting recombinant yeast expression plasmid can then be transformed by standard methods into the cells of an appropriate, compatible yeast host, which cells are able to express the recombinant protein from the recombinant yeast expression vector. Preferably, a host yeast cell transformed with such a recombinant expression vector is also able to process the fusion protein to provide an active KI polypeptide useful in the methods and compositions of the invention. A preferred yeast host for producing recombinant Kunitz domain polypeptides and KI polypeptides comprising such Kunitz domains is Pichia

As noted above, KI polypeptides that are useful in the methods and compositions described herein can comprise a Kunitz domain polypeptide described herein. Some KI polypeptides can comprise an additional flanking sequence, preferably of one to six amino acids in length, at the amino and/or carboxy-terminal end, provided such additional amino acids do not significantly diminish kallikrein binding affinity or kallikrein inhibition activity so as to preclude use in the methods and compositions described herein. Such additional amino acids can be deliberately added to express a KI polypeptide in a particular recombinant host cell or can be added to provide an additional function, e.g., to provide a peptide to link the KI polypeptide to another molecule or to provide an affinity moiety that facilitates purification of the polypeptide. Preferably, the additional amino acid(s) do not include cysteine, which could interfere with the disulfide bonds of the Kunitz domain.

An example of a preferred Kunitz domain polypeptide Alternatively, Kunitz domain polypeptides and KI 50 useful in the methods and compositions of the invention has the amino acid sequence of residues 3-60 of SEQ ID NO:2. When expressed and processed in a yeast fusion protein expression system (e.g., based on the integrating expression plasmid pHIL-D2), such a Kunitz domain polypeptide retains an additional amino terminal Ghu-Ala dipeptide from the fusion with the mat.alpha. prepro leader peptide sequence of S. cerevisiae. When secreted from the yeast host cell, most of the leader peptide is processed from the fusion protein to yield a functional KI polypeptide (referred to herein as "PEP-1") having the amino acid sequence of SEQ ID NO:2 (see boxed region in FIG. 2).

Particularly preferred KI polypeptides useful in the methods and compositions described herein have a binding affinity for kallikrein that is on the order of 1000 times higher than that of aprotinin, which is currently approved for use in CABG procedures to reduce blood loss. The surprisingly high binding affinities of such KI polypeptides

described herein indicate that such KI polypeptides exhibit a high degree of specificity for kallikrein to the exclusion of other molecular targets (see Table 1, below). Thus, use of such polypeptides according to the invention reduces much of the speculation as to the possible therapeutic targets in a 5 patient. The lower degree of specificity exhibited by, for example, aprotinin, leads to possible pleiotropic side effects and ambiguity as to its therapeutic mechanism.

The polypeptides defined by, for example, SEQ ID NO:1 contain invariant positions, e.g., positions 5, 14, 30, 51 and 10 55 can be Cys only. Other positions such as, for example, positions 6, 7, 8, 9, 20, 24, 25, 26, 27, 28, 29, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53 and 54 can be any amino acid (including non-naturally occurring amino acids). In a parcorrespond to that of a native sequence (e.g., SEQ ID NO:32, see FIG. 3). In a preferred embodiment, at least one variable position is different from that of the native sequence. In yet another preferred embodiment, the amino acids can each be individually or collectively substituted by 20 a conservative or non-conservative amino acid substitution. Conservative amino acid substitutions replace an amino acid with another amino acid of similar chemical structure and may have no affect on protein function. Non-conservative amino acid substitutions replace an amino acid with another 25 amino acid of dissimilar chemical structure. Examples of conserved amino acid substitutions include, for example, Asn→Asp, Arg→Lys and Ser→Thr. In a preferred embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and/or 21 of these amino acids can be indepen- 30 dently or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2.

Other positions, for example, positions 10, 11, 13, 15, 16, 17, 18, 19, 21, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45, can be any of a selected set of amino acids. Thus SEQ ID NO:1 defines a set of possible sequences. Each member of this set contains, for example, a cysteine at positions 5, 14, 30, 51 and 55, and any one of a specific set of amino acids at positions 10, 11, 13, 15, 16, 17, 18, 19, 221, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45. In a preferred embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and/or 19 of these amino acids can be independently or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2. The peptide preferably has at least 80%, at least 85%, at least 90% or at least 95% 45 identity to SEQ ID NO:2.

### Methods and Compositions

The present invention is also directed to methods for preventing or reducing ischemia. Preferred in the invention 50 are methods for preventing or reducing perioperative blood loss and/or a systemic inflammatory response (SIR) in a patient, especially associated with cardiothoracic surgery. A method for treatment involves the administration of a KI polypeptide comprising a Kunitz domain. One embodiment 55 of the method involves using a peptide containing an amino acid sequence of SEQ ID NO:1 that has an affinity for kallikrein that is approximately 1000-fold or more higher than that of a broad range serine protease, e.g., aprotinin, which is isolated from bovine lung and currently approved for use in CABG procedures (TRASYLOL®, Bayer Corporation Pharmaceutical Division, West Haven, Conn.).

Patients subjected to any of a number of surgical procedures, especially those involving extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB, and/or bone trauma, such as sternal split or hip replacement, are at risk for perioperative blood loss and inflammation.

Contact of a patient's blood with the cut surfaces of bone or of CPB equipment is sufficient to activate one or several undesirable cascade responses, including a contact activation system (CAS), which can lead to extensive perioperative blood loss requiring immediate blood transfusion, as well as a systemic inflammatory response (SIR), which, in turn, can result in permanent damage to tissues and organs. While not desiring to be limited to any particular mechanism or theory, it appears that the blood loss that occurs associated with cardiothoracic surgery, e.g., CPB, as in a CABG procedure, probably results from extensive capillary leakage, which can result in significant loss of blood that must be replaced by immediate blood transfusion.

The methods described herein are useful for preventing or ticularly preferred embodiment, one or more amino acids 15 reducing various ischemias including, for example, perioperative blood loss and SIR in a patient subjected to a surgical procedure, and especially wherein the surgical procedure requires extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB. The methods of the invention are particularly useful for preventing or reducing perioperative blood loss and/or SIR in a patient subjected to a CABG procedure requiring CPB or other cardiac surgery.

Preferred compositions for medical use comprise a KI polypeptide described herein. Such compositions useful can further comprise one or more pharmaceutically acceptable buffers, carriers, and excipients, which can provide a desirable feature to the composition including, but not limited to, enhanced administration of the composition to a patient, enhanced circulating half-life of the KI polypeptide of the composition, enhanced compatibility of the composition with patient blood chemistry, enhanced storage of the composition, and/or enhanced efficacy of the composition upon administration to a patient. In addition to a KI polypeptide described herein, compositions can further comprise one or more other pharmaceutically active compounds that provide an additional prophylactic or therapeutic benefit to a patient of an invasive surgical procedure.

Compositions useful in the methods of the invention comprise any of the Kunitz domain polypeptides or KI polypeptides comprising such Kunitz domain polypeptides described herein. Particularly preferred are KI polypeptides comprising a Kunitz domain polypeptide having a 58-amino acid sequence of amino acids 3-60 of SEQ ID NO:2. An example of such a particularly preferred KI polypeptide useful in the methods and compositions of the invention is the PEP-1 KI polypeptide having the 60-amino acid sequence of SEQ ID NO:2. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2 is provided in SEQ ID NO:3 (see, e.g., nucleotides 309-488 in FIG. 2). It is understood that based on the known genetic code, the invention also provides degenerate forms of the nucleotide sequence of SEQ ID NO:3 by simply substituting one or more of the known degenerate codons for each amino acid encoded by the nucleotide sequence. Nucleotides 7-180 of SEQ ID NO:3, and degenerate forms thereof, encode the non-naturally occurring Kunitz domain polypeptide having the 58-amino acid sequence of amino acids 3-60 of SEQ ID

Any of a variety of nucleic acid molecules can comprise 60 the nucleotide sequence of nucleotides 7-180 of SEQ ID NO:3, degenerate forms, and portions thereof, including but not limited to, recombinant phage genomes, recombinant mammalian viral vectors, recombinant insect viral vectors, yeast mini chromosomes, and various plasmids. Such plasmids include those used to clone and/or express such nucleotide coding sequences. Expression vectors provide a promoter, which can be operably linked to a particular

nucleotide sequence and an appropriate host cell, which is able to transcribe the particular nucleotide coding sequence into a functional messenger RNA (mRNA) and also translate the mRNA into the corresponding polypeptide. A polypeptide so produced can then be isolated from the host cell. 5 Nucleic acid molecules comprising a nucleic acid sequence encoding a Kunitz domain or KI polypeptide described herein can be made by standard nucleic acid synthesis methods, recombinant DNA methodologies, polymerase chain reaction (PCR) methods, and any combination thereof. 10

Perioperative Blood Loss and Reduced Heart Bloodflow

Due to the many advances in medicine, a number of highly invasive surgical procedures are carried out each day that result in blood loss, or place patients at a high risk for 15 blood loss. Such patients must be carefully monitored to restore and maintain normal blood supply and hemostasis, and they may need blood transfusions. Surgical procedures that involve blood loss include those involving extra-corporeal circulation methods such as cardiothoracic surgery, e.g., CPB. In such methods, a patient's heart is stopped and the circulation, oxygenation, and maintenance of blood volume are carried out artificially using an extra-corporeal circuit and a synthetic membrane oxygenator. These techniques are commonly used during cardiac surgery. Additionally, it is apparent that surgery involving extensive trauma to bone, such as the sternal split necessary in CABG or hip replacement procedures, is also associated with activation of the CAS, which can result in a variety of disruptions in the blood and vasculature.

Atherosclerotic coronary artery disease (CAD) causes a narrowing of the lumen of one or several of the coronary arteries; this limits the flow of blood to the myocardium (i.e., the heart muscle) and can cause angina, heart failure, and myocardial infarcts. In the end stage of coronary artery 35 atherosclerosis, the coronary circulation can be almost completely occluded, causing life threatening angina or heart failure, with a very high mortality. CABG procedures may be required to bridge the occluded blood vessel and restore blood to the heart; these are potentially life saving. CABG 40 procedures are among the most invasive of surgeries in which one or more healthy veins or arteries are implanted to provide a "bypass" around the occluded area of the diseased vessel. CABG procedures carry with them a small but important perioperative risk, but they are very successful in 45 providing patients with immediate relief from the mortality and morbidity of atherosclerotic cardiovascular disease. Despite these very encouraging results, repeat CABG procedures are frequently necessary, as indicated by a clear increase in the number of patients who eventually undergo 50 second and even third procedures; the perioperative mortality and morbidity seen in primary CABG procedures is increased in these re-do procedures.

There have been improvements in minimally invasive nearly all CABG procedures performed for valvular and/or congenital heart disease, heart transplantation, and major aortic procedures, are still carried out on patients supported by CPB. In CPB, large cannulae are inserted into the great vessels of a patient to permit mechanical pumping and 60 oxygenation of the blood using a membrane oxygenator. The blood is returned to the patient without flowing through the lungs, which are hypoperfused during this procedure. The heart is stopped using a cardioplegic solution, the patient cooled to help prevent brain damage, and the peripheral 65 circulating volume increased by an extracorporeal circuit, i.e., the CPB circuit, which requires "priming" with donor

blood and saline mixtures are used to fill the extracorporeal circuit. CPB has been extensively used in a variety of procedures performed for nearly half a century with successful outcomes. The interaction between artificial surfaces, blood cells, blood proteins, damaged vascular endothelium, and extravascular tissues, such as bone, disturbs hemostasis and frequently activates the CAS, which, as noted above, can result in a variety of disruptions in the blood and vasculature. Such disruption leads to excess perioperative bleeding, which then requires immediate blood transfusion. A consequence of circulating whole blood through an extracorporeal circuit in CPB can also include the systemic inflammatory response (SIR), which is initiated by contact activation of the coagulation and complement systems. Indeed, much of the morbidity and mortality associated with seemingly mechanically successful CPB surgical procedures is the result of the effects of activating coagulation, fibrinolysis, or complement systems. Such activation can damage the pulmonary system, leading to adult respi-20 ratory distress syndrome (ARDS), impairment of kidney and splanchnic circulation, and induction of a general coagulopathy leading to blood loss and the need for transfusions. In addition to the dangers of perioperative blood loss, additional pathologies associated with SIR include neurocognitive deficits, stroke, renal failure, acute myocardial infarct, and cardiac tissue damage.

Blood transfusions also present a significant risk of infection and elevate the cost of CABG or other similar procedures that require CPB. In the absence of any pharmaco-30 logical intervention, three to seven units of blood must typically be expended on a patient, even with excellent surgical techniques. Accordingly, there is considerable incentive for the development of new and improved pharmacologically effective compounds to reduce or prevent perioperative bleeding and SIR in patients subjected to CPB and CABG procedures.

Administration and Dosing Considerations for KI Polypep-

KI polypeptides described herein can be administered to a patient before, during, and/or after a surgical procedure in a pharmaceutically acceptable composition. The term "pharmaceutically acceptable" composition refers to a non-toxic carrier or excipient that may be administered to a patient, together with a compound of this invention, and wherein the carrier or excipient not destroy the biological or pharmacological activity of the composition. KI polypeptides described herein can be administered locally or systemically by any suitable means for delivery of a kallikrein inhibitory amount of the KI polypeptides to a patient including but not limited to systemic administrations such as, for example, intravenous and inhalation. Parenteral administration is particularly preferred.

For parenteral administration, the polypeptides can be surgical techniques for uncomplicated CAD. However, 55 injected intravenously, intramuscularly, intraperitoneally, or subcutaneously. Intravenous administration is preferred. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Other pharmaceutically acceptable carriers include, but are not limited to, sterile water, saline solution, and buffered saline (including buffers like phosphate or acetate), alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, paraffin, etc. Where necessary, the composition can also include a solubilizing agent and a local anaesthetic such as lidocaine to ease pain at the site of the injection, preservatives, stabilizers, wetting agents, emulsifiers, salts, lubricants, etc. as long as they do not react

deleteriously with the active compounds. Similarly, the composition can comprise conventional excipients, e.g., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active 5 compounds. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent in activity 10 units. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade "water for injection" or saline. Where the composition is to be administered by injection, an ampoule of sterile water for injection or saline 15 can be provided so that the ingredients can be mixed prior to administration.

Preferably, the methods of the invention comprise administering a KI polypeptide to a patient as an intravenous infusion according to any approved procedure. Thus, a KI 20 polypeptide described herein can be administered to a patient subjected to a CABG procedure at the times similar to those currently used in approved protocols for administering aprotinin and in an amount necessary to provide a patient with a required number or concentration of kallikrein 25 inhibitory units (KIU). According to the invention, a KI polypeptide described herein can also be administered to a patient in the immediate postoperative period, when bleeding abnormalities can occur as a consequence of downstream effects of SIR. For example, in a procedure involving 30 CPB, a KI polypeptide described herein can be administered to a patient as an initial loading dose, e.g., an effective amount over the course of a convenient time, such as 10 minutes, prior to induction of anesthesia. Then, at induction injected into the CPB priming fluid ("pump prime volume"). The patient can then be placed on a continuous and controlled intravenous infusion dose for the duration of the surgical procedure, and after the procedure if indicated.

Currently there are two regimens approved in the United 40 States for administering aprotinin to a patient undergoing a CABG procedure (see, product label and insert for TRA-SYLOL®, Bayer Corporation Pharmaceutical Division, West Haven, Conn.). One such approved regimen uses a 2 million KIU intravenous loading dose, 2 million KIU into 45 the pump prime volume, and 500,000 KIU per hour of surgery. Another approved regimen uses 1 million KIU intravenous loading dose, 1 million KIU into the pump prime volume, and 250,000 KIU per hour of surgery. As these regimens are based on KIU, the regimens are readily 50 adapted to any KI polypeptide described herein once the specific activity and KIU of a particular KI polypeptide has been determined by standard assays. Owing to the enhanced binding affinity and inhibitory activity in representative KI polypeptides described herein relative to aprotinin, it is 55 expected that such compositions and methods of the invention are likely to require fewer milligrams (mg) per patient to provide a patient with the required number or concentration of KIU.

Several considerations regarding dosing with a KI 60 polypeptide in methods of the invention can be illustrated by way of example with the representative PEP-1 KI polypeptide of the invention having the amino sequence of SEQ ID NO:2 (molecular weight of 7,054 Daltons).

Table 1, below, provides a comparison of the affinity 65 (K.sub.i,app) of the PEP-1 KI polypeptide for kallikrein and eleven other known plasma proteases.

1TABLE 1 Aprotinin Protease Substrate PEP-1 K.sub.i,app (pM) K.sub.i,app (pM) human plasma kallikrein 44 3.0 .times. 10.sup.4 human urine kallikrein>1 .times. 10.sup.8 4.0 .times. 10.sup.3 porcine pancreatic kallikrein 2.7 .times. 10.sup.7 550 human C1r, activated>2.0 .times. 10.sup.8>1.0 .times. 10.sup.7 human C1s, activated>2.0 .times. 10.sup.7>1.0 .times. 10.sup.8 human plasma factor XIa 1.0 .times. 10.sup.4 ND human plasma factor XIIa>2.0 .times. 10.sup.7>1.0 .times. 10.sup.8 human plasmin 1.4 .times. 10.sup.5 894 human pancreatic trypsin>2 .times. 10.sup.7 ND human pancreatic chymotrypsin>2.0 .times. 10.sup.7 7.3 .times. 10.sup.5 human neutrophil elastase>2.0 .times. 10.sup.7 1.7 .times. 10.sup.6 human plasma thrombin>2.0 .times. 10.sup.7>1.0 .times. 10.sup.8 ND=not determined

Clearly, the PEP-1 KI polypeptide is highly specific for human plasma kallikrein. Furthermore, the affinity (K.sub.i, app) of PEP-1 for kallikrein is 1000 times higher than the affinity of aprotinin for kallikrein: the K.sub.i,app of PEP-1 for kallikrein is about 44 pM (Table 1), whereas the K.sub.i, app of aprotinin for kallikrein is 30,000 pM. Thus, a dose of PEP-1 could be approximately 1000 times lower than that used for aprotinin on a per mole basis. However, consideration of several other factors may provide a more accurate estimation of the dose of PEP-1 required in practice. Such factors include the amount of kallikrein activated during CPB in a particular patient, the concentration of kallikrein required to elicit an SIR, and the bioavailability and pharmacological distribution of PEP-1 in a patient. Nevertheless, use of a KI polypeptide in methods according to the invention and provided in doses currently approved for the use of aprotinin is still expected to provide significant improvements over the current use of the less specific, lower affinity, bovine aprotinin.

For example, the total amount of circulating prekallikrein in plasma is estimated at approximately 500 nM (Silverberg, M. et al., "The Contact System and Its Disorders," in Blood: Principles and Practice of Hematology, Handin, R. et al., "B Lippincott Co., Philadelphia, 1995). If all of the surgical procedure, and after the procedure if indicated.

Currently there are two regimens approved in the United States for administering aprotinin to a patient undergoing a CABG procedure (see, product label and insert for TRA-SYLOL®, Bayer Corporation Pharmaceutical Division,

Another factor to consider is the threshold concentration of kallikrein required to induce a SIR in a patient. If the concentration of active kallikrein must be maintained below, e.g., 1 nM, then owing to its high affinity for kallikrein, PEP-1 offers a significant advantage over aprotinin in the amount of protein that would be required to inhibit SIR. In particular, a concentration of PEP-1 of 1 nM would inhibit 99.6% of kallikrein present at 1 nM (i.e., only 0.4 pM free kallikrein remaining in the blood), whereas, an aprotinin concentration of 1 nM would only inhibit 24.5% of the kallikrein present at 1 nM. For aprotinin to inhibit 99% of the kallikrein at 1 nM, an aprotinin concentration in the plasma of at least 3 mu.M is required (i.e., 3000 times higher concentration than for PEP-1).

For a patient undergoing CPB, an initial clinical dose of PEP-1 can be estimated from a recommended dose regimen of aprotinin (1 times. 10.sup.6 KIU) mentioned above. Aprotinin is reported in a package insert to have as specific inhibitory activity of 7143 KIU/mg determined using a dog blood pressure assay. Therefore, 1 .times. 1 .sup.6 KIU of aprotinin is equivalent to 140 mg of aprotinin (i.e., 1 .times. 10.sup.6 KIU/7143 KIU/mg=140 mg of aprotinin). In a patient having a blood plasma volume of 5 liters, 140 mg corresponds to approximately 4.3 .mu.M aprotinin (molecu-

lar weight of aprotinin is 6512 Daltons). The specific activity of aprotinin in the standard inhibitory assay used for PEP-1 is 0.4 KIU/mg of polypeptide. A dose of 140 mg would correspond to a loading dose for aprotinin of 56 KIU (140 mg.times.0.4 KIU/mg=56 KIU). In contrast, since the specific activity of the PEP-1 KI polypeptide is 10 KIU/mg in the standard inhibition assay, a dose of only 5.6 mg of PEP-1 would be required to provide the number of KIUs equivalent to 140 mg of aprotinin. In a patient with a plasma volume of 5 liters, this corresponds to about 160 nM PEP-1 (molecular weight of PEP-1 is 7054 Daltons), although a higher dose of the PEP-1 KI polypeptide can be required if all of the plasma kallikrein (500 nM) is activated and/or if this KI polypeptide is poorly distributed in a patient.

Furthermore, the KI polypeptides can be non-naturally 15 occurring, and they can be produced synthetically or recombinantly, as noted above, thereby avoiding potential contamination of transmissible diseases that can arise during isolation of a protein from a natural animal source, such as in the case of aprotinin, which is isolated from bovine lung. 20 Increasingly important to administrative and public acceptance of a treatment or pharmaceutical composition comprising a polypeptide is the avoidance of possible contamination with and transmission to human patients of various pathological agents. Of particular interest for the safety of 25 proteins isolated from a bovine tissue is the elimination of the possible risk of exposure to viral mediated diseases, bacterial mediated diseases, and, especially, transmissible bovine spongiform encephalopathies.

As variants of the Kunitz domain 1 of the human LACI 30 protein, fewer side effects are expected from administering the KI polypeptides to patients than for aprotinin, which is a bovine protein that is documented to cause anaphylactic and anaphylactoid responses in patients, especially in repeat administrations, such as second time CABG procedures. 35 Additionally, the highly specific binding of the KI polypeptides described herein to kallikrein will effectively limit or eliminate the thrombotic tendencies observed with aprotinin, and reduce the problems observed with graft patency following CABG procedures.

The invention will be further described with reference to the following non-limiting examples. The teachings of all the patents, patent applications and all other publications and websites cited herein are incorporated by reference in their entirety.

### **EXEMPLIFICATION**

### Example 1

### A Representative KI Polypeptide

A non-naturally occurring, KI polypeptide useful in the compositions and methods of the invention was identified as a kallikrein binding polypeptide displayed on a recombinant phage from a phage display library. PEP-1 has the following amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2). The molecular weight of PEP-1 is 7,054 Daltons.

The nucleotide sequence (SEQ ID NO:3) encoding the PEP-1 amino acid sequence (SEQ ID NO:2), was derived from a peptide that was isolated and sequenced by standard 65 methods determined from the recombinant phage DNA. PEP-1 was produced in amounts useful for further charac-

terization as a recombinant protein in His4.sup.-phenotype host cells of yeast strain *Pichia pastoris*.

#### Example 2

Construction of a Recombinant Plasmid to Express KI Polypeptides

The initial plasmid, pHIL-D2, is ampicillin resistant and contains a wild-type allele of His4 from *P. pastoris*. The final DNA sequence comprising the coding sequence for the mat.alpha. Prepro-PEP-1 fusion protein in the recombinant expression plasmid pPIC-K503 is shown in FIG. 2. The DNA sequence of pHIL-D2 was modified to produce pPIC-K503, as follows:

- 1. The BstBI site in the 3' AOX1 region of pHIL-D2, located downstream of the His4 gene, was removed by partial restriction digestion, fill-in, and ligation, altering the sequence from TTCGAA (SEQ ID NO:23) to TTCGCGAA (SEQ ID NO:24). This modification was made to facilitate and direct the cloning of the expression cassette into the plasmid.
- The AatII site bearing the bla gene located downstream of His4 was removed by restriction digestion, fill-in, and ligation modifying the sequence from GACGTC (SEQ ID NO:25) to GACGTACGTC (SEQ ID NO:26). This modification was made to facilitate the cloning of expression cassettes having AatlI sites into the plasmid. The DNA encoding PEP-1 was synthesized based on the nucleotide sequence from the original kallikrein-binding display phage and consisted of 450 base pairs (bp). The final DNA sequence of the insert in the pHIL-D2 plasmid is flanked by a 5' AOX1 sequence and a 3' AOX1 sequence (portions of which are shown in FIG. 2) and encode a fusion protein comprising the mat.alpha. prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 KI polypeptide. The signal peptide was added to facilitate the secretion of PEP-1 from the yeast host cells. The oligonucleotides to form the insert were synthesized and obtained commercially (Genesis Labs, The Woodlands, Tex.), and the insert was generated by polymerase chain reaction (PCR). The linked synthetic DNA encoding the mat.alpha. prepro/PEP-1 fusion protein was then incorporated by ligation into the modified pHIL-D2 plasmid between the BstBI and EcoRI sites.

The ligation products were used to transform Escherichia coli strain XL1 Blue. A PCR assay was used to screen E. coli transformants for the desired plasmid construct. DNA from cell extracts was amplified by PCR using primers containing the 5' AOX1 and 3' AOX1 sequences (see above and FIG. 2). PCR products of the correct number of base pairs were sequenced. In addition, approximately 20-50 bp on either side of the cloning sites were sequenced, and the predicted sequence was obtained. The final DNA sequence of the insert in the pHIL-D2 plasmid (to yield plasmid pPIC-K503) is shown in FIG. 2 along with portions of flanking 5' and 3' AOX1 sequences and corresponding amino acid sequence of the fusion protein comprising the mat.alpha. prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 KI polypeptide. A transformant with the desired expression plasmid construct, plasmid pPIC-K503, was selected for preparing yeast cell lines for routine production of PEP-1.

Manufacture of PEP-1 from Recombinant Yeast Cell Line

Spheroplasts of P. pastoris GS115 having the His4.sup.- 5 phenotype were transformed with the expression plasmid pPIC-K503 (above) following linearization of the plasmid at the SacI site and homologous recombination of the plasmid DNA into the host 5' AOX1 locus. The phenotype of the production strain is His4.sup.+. The entire plasmid was inserted into the 5' AOX1 genomic sequence of the yeast.

Isolates from the transformation were screened for growth in the absence of exogenous histidine with methanol as the sole carbon source. Greater than 95% of the transformants 15 retained the wild-type ability to grow with methanol as the sole carbon source, thereby demonstrating that the plasmid had been inserted into the host genome by homologous recombination rather than transplacement. These transformants did not require exogenous histidine for growth, thereby demonstrating that the plasmid had integrated into the host genome. Selected colonies were cloned. Small culture expression studies were performed to identify clones secreting the highest levels of active PEP-1 into the culture 25 medium. PEP-1 secretion levels in clarified culture supernatant solutions were quantified for PEP-1 levels by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and evaluated for kallikrein inhibition. A yeast clone was selected for PEP-1 production based on its high level of 30 PEP-1 expression among cultures sampled.

Master and working cell banks of P. pastoris producing PEP-1 were prepared commercially (MDS Pharma Services, Bothell, Wash.). A standard production of PEP-1 in yeast comprised three steps as follows: (1) preparation of the seed culture, (2) fermentation, and (3) recovery of the culture.

The seed culture step consisted of the inoculation of six flasks (300 mL each) containing sterile inoculum broth (yeast nitrogen base, potassium phosphate, and glycerol, 40 pH=5) with the contents of a single vial of a working cell bank of P. pastoris producing PEP-1. Flasks were inoculated in an orbital shaker (300 rpm) for approximately 13 hours at 30.degree. C.+-0.2.degree. C.

Fermentations were performed in a closed 100 liter Braun fermenter filled with sterile broth. Each fermentation was initiated with the transfer of the contents of the six seed culture flasks to the fermenter. After approximately 24 hours, the glycerol in the fermenter became exhausted and 50 fluorescence following kallikrein-mediated cleavage of a additional glycerol was added for approximately 8 additional hours.

A mixed feed phase, which lasted approximately 83 hours, was then initiated by the addition of a glycerol and methanol feed. At the end of this time, the fermentation was 55 terminated, and the fermenter contents were diluted with purified water. The purification and processing of PEP-1 consisted of five steps as follows: (I) expanded bed chromatography, (2) cation exchange chromatography, (3) hydrophobic interaction chromatography (HIC), (4) ultrafiltration and diafiltration, and (5) final filtration and packag-

The initial purification step consisted of expanded bed chromatography. The diluted fermenter culture was applied 65 to the equilibrated column packed with Streamline SP resin (Amersham Pharmacia Streamline 200 chromatography col

umn, Amersham Pharmacia, Piscataway, N.J.). The column was then washed (50 mM acetic acid, pH=3.0-3.5) in an up-flow mode to flush the yeast cells from the expanded bed. The top adaptor was raised above the expanded bed enhance washing. The flow was stopped and the bed was allowed to settle. The adaptor was moved down so that it was slightly above the settled bed. The direction of the flow was reversed. The effluent was collected. Washing was continued in a downward mode using 50 mM sodium acetate, pH 4.0. The effluent was collected. PEP-1 was eluted from the column using 50 mM sodium acetate, pH 6.0. The eluate was collected in a 50 liter container. The eluate was then filtered through a 0.22.mu. filter into a clean container located in the purification site. Additional samples were collected for the determination of PEP-1 concentration. A cation exchange chromatography step was then performed using the filtered eluate from the expanded bed column. PEP-1 was eluted from the column using 15 mM trisodium citrate, pH 6.2.

Additional proteins were removed from the PEP-1 preparation by hydrophobic interaction chromatography (HIC). Prior to HIC, the eluate from the cation exchange column was diluted with ammonium sulfate. The eluate was applied to the column, and the PEP-1 was eluted using ammonium sulfate (0.572 M) in potassium phosphate (100 mM), pH 7.0. The eluate was collected in fractions based on A280 values. All fractions were collected into sterile, pre-weighed PETG bottles.

Selected fractions were pooled into a clean container. The pool was concentrated by ultrafiltration. The concentrated PEP-1 preparation was immediately diafiltered against ten volumes of PBS, pH 7.0.

A final filtration step was performed prior to packaging in order to minimize the bioburden in the bulk PEP-1. The bulk solution was filtered through a 0.22.mu. filter and collected into a sterile, pre-weighed PETG bottle. A sample was removed for lot release testing. The remainder of the bulk was dispensed aseptically into sterile PETG bottles and stored at -20.degree. C.

### Example 4

Kallikrein Inhibition Assay

A kinetic test was used to measure inhibitory activity of KI polypeptides, such as PEP-1. The kinetic assay measures substrate, prolylphenylalanylarginyl amino methyl coumarin. A known amount of kallikrein was incubated with a serially diluted KI polypeptide reference standard or serially diluted KI polypeptide test samples, in a suitable reaction buffer on a microtiter plate. Each sample was run in triplicate. The substrate solution was added, and the plate read immediately using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. At least two each of the reference standard and sample curves were required to have an R-squared value of 0.95 to be considered valid.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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1 10 15
Ser Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ile Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
```

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<210> SEQ ID NO 8
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 8
 Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
1 10 15
 Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
 Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 \hspace{1.5cm} 40 \hspace{1.5cm} 45 \hspace{1.5cm}
 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
 <210> SEQ ID NO 9
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 9
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Gly 1 5 10 15
Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
<210> SEQ ID NO 10
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 10
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Lys Gly
1 5 10 15
Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg GIn Cys Glu Glu 20 25 30
Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
<210> SEQ ID NO 11
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 11
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly
```

Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu

20 Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp 50 55 <210> SEQ ID NO 12 <211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Isolated Binding Peptide <400> SEQUENCE: 12 Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 45 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50
55 <210> SEQ ID NO 13 <211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Isolated Binding Peptide <400> SEQUENCE: 13 Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly
1 5 10 15 Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg GIn Cys Glu Glu 20 25 30Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp <210> SEQ ID NO 14 <211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Isolated Binding Peptide <400> SEQUENCE: 14 Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly
1 5 10 15 Ala Gln Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45Glu Glu Cys Lys Lys Met Cys Thr Arg Asp <210> SEQ ID NO 15 <211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 15
 Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Ser Cys Arg Ala
1 5 10 15
 Ala His Leu Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
 Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45
 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
 <210> SEQ ID NO 16
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 16
Met His Ser Phe Cys Ala Phe Lys Ala Glu Gly Gly Ser Cys Arg Ala
1 5 10 15
Ala His Gln Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
<210> SEQ ID NO 17
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 17
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Gly
1 5 10 15
Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
<210> SEQ ID NO 18
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 18
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Arg Gly 1 5 10 15
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Ala Leu Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45

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Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
  <210> SEQ ID NO 19
  <211> LENGTH: 58
  <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 19
 Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Asn Cys Arg Gly
1 5 10 15
 Asn Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 25 30
 Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 \hspace{1.5cm} 40 \hspace{1.5cm} 45 \hspace{1.5cm}
 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
 <210> SEQ ID NO 20
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Isolated Binding Peptide
 <400> SEQUENCE: 20
Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Arg Cys Arg Gly 1 5 10 15
As His Gln Arg Phe Phe Phe As Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
<210> SEQ ID NO 21
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 21
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Ala
1 10 15
Ile Gln Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
<210> SEQ ID NO 22
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide
<400> SEQUENCE: 22
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Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Arg Gly
1 5 10 15
 Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
 Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
 Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
 <210> SEQ ID NO 23
 <211> LENGTH: 6
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Modified Cloning Site
<400> SEQUENCE: 23
ttcgaa
                                                                          6
<210> SEQ ID NO 24
 <211> LENGTH: 8
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site
<400> SEQUENCE: 24
ttcgcgaa
                                                                          8
<210> SEQ ID NO 25
<211> LENGTH: 6
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site
<400> SEQUENCE: 25
gacgtc
                                                                         6
<210> SEQ ID NO 26
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site
<400> SEQUENCE: 26
gacgtacgtc
                                                                        10
<210> SEQ ID NO 27
<211> LENGTH: 548
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide Sequence of Pusion Protein
<400> SEQUENCE: 27
cgacttttaa cgacaacttg agaagatcaa aaaacaacta attattcgaa acgatgagat
                                                                        60
toccatetat etteactget gttttgtteg etgetteete tgetttgget getecagtta
acaccactac tgaagacgag actgctcaaa ttcctgctga ggctgtcatc ggttactctg
                                                                       180
acttggaagg tgacttcgac gtcgctgttt tgccattctc taactctact aacaacqqtt
                                                                      240
```

420

480

540

548

-continued tgttgttcat caacactacc atcgcttcta tcgctgctaa ggaggaaggt gtttccctcg agaagagaga ggctatgcac tctttctgtg ctttcaaggc tgacgacggt ccgtgcagag ctgctcaccc aagatggttc ttcaacatct tcacgcgtca atgcgaggag ttcatctacg gtggttgtga gggtaaccaa aacagattcg agtctctaga ggagtgtaag aagatgtgta ctagagacta gtaagaattc gccttagaca tgactgttcc tcagttcaag ttgggcactt acgagaag <210> SEQ ID NO 28 <211> LENGTH: 145 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Fusion Protein <400> SEQUENCE: 28 Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser 1 5 10 15 Ala Leu Ala Ala Pro Val Asn Thr Thr Glu Asp Glu Thr Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe 35 40 45 Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu 50 60 Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val 65 70 75 80 Ser Leu Glu Lys Arg Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala 85 90 95 Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile 100 105 110 Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn 115 120 125 Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg 130 135 140 qaA 145 <210> SEQ ID NO 29 <211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: BPTI Sequence <400> SEQUENCE: 29 Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly Pro Cys Lys Ala 1 5 10 15 Arg Ile Ile Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gln Thr 20 25 30

Glu Asp Cys Met Arg Thr Cys Gly Gly Ala

Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg Asn Asn Phe Lys Ser Ala 35 40 45

<sup>&</sup>lt;210> SEQ ID NO 30

<sup>&</sup>lt;211> LENGTH: 58

<sup>&</sup>lt;212> TYPE: PRT

<sup>&</sup>lt;213> ORGANISM: Artificial Sequence

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<220> FEATURE:
  <223> OTHER INFORMATION: ITI-D1 Sequence
  <400> SEQUENCE: 30
  Lys Glu Asp Ser Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Met Gly
1 5 10 15
  Met Thr Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Glu Thr 20 25 30
  Phe Gln Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
 Lys Glu Cys Leu Gln Thr Cys Arg Thr Val
50 55
  <210> SEQ ID NO 31
  <211> LENGTH: 58
  <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
  <220> FEATURE:
 <223> OTHER INFORMATION: ITI-D2 Sequence
 <400> SEQUENCE: 31
 Thr Val Ala Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Arg Ala
1 5 10 15
 Phe Ile Gln Leu Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu
20 25 30
 Phe Pro Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu
35 40 45
 Lys Glu Cys Arg Glu Tyr Cys Gly Val Pro
50 55
 <210> SEQ ID NO 32
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: LACI-D1 Sequence
 <400> SEQUENCE: 32
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala
1 5 10 15
Ile Met Lys Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 25 30
Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
<210> SEQ ID NO 33
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LACI-D2 Sequence
<400> SEQUENCE: 33
Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Pro Gly Ile Cys Arg Gly
1 5 10 15
Tyr Ile Thr Arg Tyr Phe Tyr Asn Asn Gln Thr Lys Gln Cys Glu Arg
20 25 30
```

Phe Lys Tyr Gly Gly Cys Leu Gly Asn Met Asn Asn Phe Glu Thr Leu 35 40 45

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Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly
       50
  <210> SEQ ID NO 34
  <211> LENGTH: 58
  <212> TYPE: PRT
  <213> ORGANISM: Artificial Sequence
  <220> FEATURE:
  <223> OTHER INFORMATION: LACI-D3 Sequence
  <400> SEQUENCE: 34
 Gly Pro Ser Trp Cys Leu Thr Pro Ala Asp Arg Gly Leu Cys Arg Ala 1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15
 As Glu As Arg Phe Tyr Tyr As Ser Val Ile Gly Lys Cys Arg Pro 20 25 30
 Phe Lys Tyr Ser Gly Cys Gly Gly Asn Glu Asn Asn Phe Thr Ser Lys 35 40 45
 Gln Glu Cys Leu Arg Ala Cys Lys Lys Gly
50 55
 <210> SEQ ID NO 35
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: HKI B9 Sequence
 <400> SEQUENCE: 35
 Leu Pro Asn Val Cys Ala Phe Pro Met Glu Lys Gly Pro Cys Gln Thr 1 5 10 15
 Tyr Met Thr Arg Trp Phe Phe Asn Phe Glu Thr Gly Glu Cys Glu Leu
20 25 30
Phe Ala Tyr Gly Gly Cys Gly Gly Asn Ser Asn Asn Phe Leu Arg Lys 35 40 45
Glu Lys Cys Glu Lys Phe Cys Lys Phe Thr
50 55
 <210> SEQ ID NO 36
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: C alpha 3 Sequence
<400> SEQUENCE: 36
Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Asp 1 10 15
Phe Ile Leu Lys Trp Tyr Tyr Asp Pro Asn Thr Lys Ser Cys Ala Arg
20 25 30
Phe Trp Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly Ser Gln 35 40 45
Lys Glu Cys Glu Lys Val Cys Ala Pro Val
<210> SEQ ID NO 37
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TFPI-2 D1 Sequence
<400> SEQUENCE: 37
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Asn Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys Arg Ala 1 5 10 15
 Leu Leu Leu Arg Tyr Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys Arg Gln 20 25 30
 Phe Leu Tyr Gly Gly Cys Glu Gly Asn Ala Asn Asn Phe Tyr Thr Trp
 Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile
 <210> SEQ ID NO 38
 <211> LENGTH: 61
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: TFPI-2 D2 Sequence
 <400> SEQUENCE: 38
Val Pro Lys Val Cys Arg Leu Gln Val Ser Val Asp Asp Gln Cys Glu
Gly Ser Thr Glu Lys Tyr Phe Phe Asn Leu Ser Ser Met Thr Cys Glu
20 25 30
Lys Phe Phe Ser Gly Gly Cys His Arg Asn Arg Ile Glu Asn Arg Phe 35 40 45
Pro Asp Glu Ala Thr Cys Met Gly Phe Cys Ala Pro Lys
<210> SEQ ID NO 39
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TFPI-2 D3 Sequence
<400> SEQUENCE: 39
Ile Pro Ser Phe Cys Tyr Ser Pro Lys Asp Glu Gly Leu Cys Ser Ala
1 5 10 15
Asn Val Thr Arg Tyr Tyr Phe Asn Pro Arg Tyr Arg Thr Cys Asp Ala
Phe Thr Tyr Thr Gly Cys Gly Gly Asn Asp Asn Asn Phe Val Ser Arg
Glu Asp Cys Lys Arg Ala Cys Ala Lys Ala
50 55
<210> SEQ ID NO 40
<211> LENGTH: 59
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: APP-I Sequence
<400> SEQUENCE: 40
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4400> SEQUENCE: 40

Arg Asn Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg

Ala Met Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala 20 25 30

Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr 35 40 45

Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala 50 55

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<210> SEQ ID NO 41
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EpiNE7 Sequence
 <400> SEQUENCE: 41
 Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly Pro Cys Val Ala
 Met Phe Pro Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gln Thr 20 \hspace{1cm} 25 \hspace{1cm} 30 \hspace{1cm}
 Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Lys Ser Ala 35 40 45
 Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
50 55
 <210> SEQ ID NO 42
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: BITI-E7-141 Sequence
 <400> SEQUENCE: 42
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Ala
1 5 10 15
Met Phe Pro Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
50 55
<210> SEQ ID NO 43
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MUTT26A Sequence
<400> SEQUENCE: 43
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Ala
1 5 10 15
Met Phe Pro Arg Tyr Phe Tyr Asn Gly Ala Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu
35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
<210> SEQ ID NO 44
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MUTQE Sequence
<400> SEQUENCE: 44
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Ala
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 Met Phe Pro Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Glu Thr
 Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu
 Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
 <210> SEQ ID NO 45
 <211> LENGTH: 58
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: MUT1619 Sequence
 <400> SEQUENCE: 45
Met Phe Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
50 55
<210> SEQ ID NO 46
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EPI-HNE-1 Sequence
<400> SEQUENCE: 46
Glu Ala Glu Ala Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly
1 5 10 15
Pro Cys Ile Ala Phe Phe Pro Arg Tyr Phe Tyr Asn Ala Lys Ala Gly
20 25 30
Leu Cys Gln Thr Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn 35 40 45
Phe Lys Ser Ala Glu Asp Cys Met Arg Thr Cys Gly Gly Ala 50 55 60
<210> SEQ ID NO 47
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EPI-HNE-2 Sequence
<400> SEQUENCE: 47
Ala Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Ile Ala Phe Phe 1 5 10 15
Pro Arg Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro 20 25 30
Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu Lys Glu
35 40 45
Cys Arg Glu Tyr Cys Gly Val Pro
```

<210> SEQ ID NO 48 <211> LENGTH: 56 <212> TYPE: PRT

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<213> ORGANISM: Artificial Sequence
  <220> FEATURE:
  <223> OTHER INFORMATION: EPI-HNE-3 Sequence
  <400> SEQUENCE: 48
  Ala Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Ile Ala Phe Phe
 Pro Arg Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro 20 25 30
 Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu Lys Glu
35
 Cys Arg Glu Tyr Cys Gly Val Pro
 <210> SEQ ID NO 49
 <211> LENGTH: 56
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: EPI-HNE-4 Sequence
 <400> SEQUENCE: 49
 Glu Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Ile Ala Phe Phe
 Pro Arg Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro 20 25 30
Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu Lys Glu 35 40 45
Cys Arg Glu Tyr Cys Gly Val Pro
50 55
 <210> SEQ ID NO 50
 <211> LENGTH: 60
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: DPI14 KR Sequence
<400> SEQUENCE: 50
Glu Ala Val Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys

1 10 15
Ile Ala Phe Phe Pro Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys 20 25 30
Ala Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Phe Asp 35 40 45
Thr Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala
<210> SEQ ID NO 51
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DPI24 KR Sequence
<400> SEQUENCE: 51
Glu Ala Asn Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys
1 10 15
Ile Ala Phe Phe Pro Arg Tyr Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys 20 25 30
```

Arg Gln Phe Leu Tyr Gly Gly Cys Glu Gly Asn Ala Asn Asn Phe Tyr

```
35
                               40
  Thr Trp Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile
  <210> SEQ ID NO 52
  <211> LENGTH: 60
  <212> TYPE: PRT
  <213> ORGANISM: Artificial Sequence
  <220> FEATURE:
  <223> OTHER INFORMATION: DPI68 KR Sequence
  <400> SEQUENCE: 52
 Glu Ala Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Pro Gly Ile Cys

1 10 15
 Ile Gly Phe Phe Pro Arg Tyr Phe Tyr Asn Asn Gln Ala Lys Gln Cys 20 25 30
 Glu Arg Phe Val Tyr Gly Gly Cys Leu Gly Asn Met Asn Asn Phe Glu
35 40 45
 Thr Leu Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly
 <210> SEO ID NO 53
 <211> LENGTH: 60
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: DPI84 KR Sequence
 <400> SEQUENCE: 53
 Glu Ala Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys
Ile Ala Phe Phe Pro Arg Trp Tyr Tyr Asp Pro Asn Thr Lys Ser Cys 20 25 30
Ala Arg Phe Val Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly 35 40 45
Ser Gln Lys Glu Cys Glu Lys Val Cys Ala Pro Val 50 60
<210> SEQ ID NO 54
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VARIANT
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 10
<223> OTHER INFORMATION: Xaa = Asp or Glu
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 11
<223> OTHER INFORMATION: Xaa = Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 13
<223> OTHER INFORMATION: Xaa = Arg, His, Pro, Asn, Ser, Thr, Ala, Gly,
     Lys or Gln
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 15
<223> OTHER INFORMATION: Kaa = Arg, Ala, Ser, Gly, Met, Asn or Gln
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Xaa Xaa Xaa Arg Xaa Phe Phe Asn Ile Phe Thr Arg Gln Cys Xaa Xaa
Phe Xaa Kaa Gly Gly Cys Xaa Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
```

45

#### What is claimed is:

- 1. An isolated polypeptide comprising the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein.
- 2. The isolated polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).
- 3. The isolated polypeptide of claim 1, wherein the polypeptide consists of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2).
- 4. The isolated polypeptide of claim 2, wherein the polypeptide consists of the amino acid sequence: Glu Ala 55 Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).

\* \* \* \* \*

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

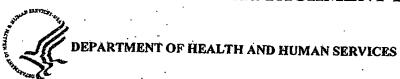
Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

Attachment D

Biologics License Approval Letter including enclosures

## ATTACHMENT D



Food and Drug Administration Silver Spring MD 20993

Our STN: BL 125277/0

BLA APPROVAL
December 1, 2009

Dyax Corporation 300 Technology Square Cambridge, MA 02139

Attention:

Nicole D'Auteuil

Senior Director, Regulatory Affairs

Dear Ms. D'Auteuil:

Please refer to your biologics license application (BLA), dated September 23, 2008, received September 23, 2008, submitted under section 351 of the Public Health Service Act (PHSA) for Kalbitor (ecallantide) injection.

We acknowledge receipt of your submissions dated December 31, 2007, March 27, September 23, October 10 and 30, November 13, 18, and 26, and December 9, 11, 15, 19 (2), 23, 24, and 31, 2008, and January 5, 9, 13, 16, 21, 23, 27, 28, and 29, February 11, 12, 13, 20, and 27, March 9, May 31, June 10 and 29, July 21, August 12 and 31, September 29, October 26 (3) and 30 (2), November 9, 16, 17, 18, 19, 23 (2), 24, 25, and 27, and December 1, 2009.

The May 31, 2009, submission constituted a complete response to our March 25, 2009, action letter.

We have completed our review of your application and are issuing Department of Health and Human Services U.S. License No. 1789 to Dyax Corporation, Cambridge, Massachusetts, under the provisions of section 351(a) of the PHSA controlling the manufacture and sale of biological products. The license authorizes you to introduce into, or deliver for introduction into, interstate commerce those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are authorized to manufacture the product Kalbitor (ecallantide) injection. Kalbitor (ecallantide) injection is indicated for the treatment of acute attacks of hereditary angioedema in patients 16 years of age and older.

Under this license, you are approved to manufacture ecallantide drug substance at Avecia Biologics in Billingham, United Kingdom. The final formulated product will be manufactured, filled, labeled, and packaged at Hollister-Stier Laboratories, LLC, Spokane, Washington. You may label your product with the proprietary name Kalbitor and market it as a sterile liquid in single-dose, 2-mL glass vials (1-mL fill), 10 mg/mL for subcutaneous injection.

You currently are not required to submit samples of future lots of Kalbitor (ecallantide) injection to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging, or labeling of Kalbitor (ecallantide) injection, or in the manufacturing facilities.

# REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

Because this biological product for this indication has an orphan drug designation, you are exempt from this requirement.

# POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a known serious risk of hypersensitivity reactions and immunogenicity, a theoretical risk of disordered coagulation, and an unexpected, serious risk of malignancy with use of Kalbitor (ecallantide) injection.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess these serious risks.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

1. A long-term, observational safety study with Kalbitor (ecallantide) in patients with hereditary angioedema to evaluate hypersensitivity, immunogenicity, and coagulation disorders. The study should include the following objectives: 1) identify predictive risk factors and develop effective screening tools to mitigate the risk of hypersensitivity and anaphylaxis; 2) correlate antibody levels with adverse events and lack of efficacy; and 3) evaluate the risk of hypercoagulability and hypocoagulability.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Protocol Submission:

. December 2009

**Study Completion Date:** 

February 2014

Final Report Submission:

August 2014

2. Establish the sensitivity and cutpoint for the anti-ecallantide neutralizing antibody assay, using immunoaffinity-purified ecallantide-specific human IgG.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Report Submission:

March 2010

3. Evaluate for cross-reactivity of anti-ecallantide antibodies with TFPI, perform studies to determine if human anti-ecallantide antibodies bind TFPI, and perform suitability studies and epitope mapping of the human anti-ecallantide antibody response if binding is observed.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Report Submission:

August 2010

4. Develop and validate anti-ecallantide and anti-P. pastoris-specific human IgE detection assays using a sensitive platform such as ECL. Such assays should be free from interference by anti-ecallantide IgG antibodies.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Method Development Reports Submission:

April 2010

Final Report Submission:

September 2010

5. A study in rats to evaluate the carcinogenic potential of Kalbitor (ecallantide). The six-month subcutaneous toxicology study with rats could serve as the basis of dose selection.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Protocol Submission:

June 2010

Study Completion Date:

September 2012

Final Report Submission:

September 2013

Submit the protocols to your IND, with a cross-reference letter to this BLA 125277. Submit all final reports to your BLA 125277. Prominently identify submissions with the following wording in bold capital letters at the top of the first page of the submission, as appropriate:

- REQUIRED POSTMARKETING PROTOCOL UNDER 505(o)
- REQUIRED POSTMARKETING FINAL REPORT UNDER 505(0)
- REQUIRED POSTMARKETING CORRESPONDENCE UNDER 505(o)

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

# POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS OF SECTION 506B

We acknowledge your written commitments as described in your letter of November 24, 2009, as outlined below:

6. The submission, as a pre-approval supplement, of an updated stability protocol for drug product that will add an accelerated or stress stability condition as part of the annual stability program. The data accumulated from this protocol will be submitted to the BLA on an annual basis.

Final Protocol Submission: January 2010

7. To evaluate the minimum fill volume required to provide appropriate dosage withdrawal and whether an adjustment to the fill volume for the drug product is necessary to reduce the likelihood that a patient will be overdosed with any excess drug product. The final study report including identification of a new fill volume, if found to be necessary, will be provided. Should the fill volume need to be changed, this report will include a proposed execution plan.

Final Report Submission: April 2010

# RISK EVALUATION AND MITIGATION STRATEGY REQUIREMENTS

Section 505-1 of the FDCA authorizes FDA to require the submission of a Risk Evaluation and Mitigation Strategy (REMS) if FDA determines that such a strategy is necessary to ensure that the benefits of the drug outweigh the risks (section 505-1(a)).

Your proposed REMS, submitted on December 1, 2009, and appended to this letter, is approved. The REMS consists of a Medication Guide, a communication plan, and a timetable for submission of assessments of the REMS.

The REMS assessment plan should include but is not limited to the following:

- a. A summary of all reported serious hypersensitivity reactions with analysis of adverse event reporting by prescriber type.
- b. Specification of measures that would be taken to increase awareness if surveys of health care providers indicate that provider awareness is not adequate.
- c. An evaluation of health care providers' understanding and patients' understanding of the serious risks of Kalbitor (ecallantide) injection.
- d. Based on the information submitted, an assessment and conclusion of whether the REMS is meeting its goals, and whether modifications to the REMS are needed.

Assessments of an approved REMS must also include, under section 505-1(g)(3)(B) and (C), information on the status of any post-approval study or clinical trial required under section 505(o) or otherwise undertaken to investigate a safety issue. You can satisfy these requirements in your REMS assessments by referring to relevant information included in the most recent annual report required under section 506B, and 21 CFR 601.70, and including any updates to the status information since the annual report was prepared. Failure to comply with the REMS assessments provisions in section 505-1(g) could result in enforcement action.

We remind you that in addition to the assessments submitted according to the timetable included in the approved REMS, you must submit a REMS assessment and may propose a modification to the approved REMS when you submit a supplemental application for a new indication for use as described in section 505-1(g)(2)(A) of FDCA.

Prominently identify the submission containing the REMS assessments or proposed modifications with the following wording in bold capital letters at the top of the first page of the submission:

BLA 125277 REMS ASSESSMENT NEW SUPPLEMENT FOR BLA 125277 PROPOSED REMS MODIFICATION REMS ASSESSMENT

NEW SUPPLEMENT (NEW INDICATION FOR USE) FOR BLA 125277 REMS ASSESSMENT PROPOSED REMS MODIFICATION (if included)

If you do not submit electronically, please send five copies of REMS-related submissions.

We request that the labeling approved today be available on your website within 10 days of product launch.

## REPORTING REQUIREMENTS

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). In addition, you should submit all reports of serious anaphylactic or hypersensitivity events within 15 days of receipt as 15-day expedited reports. You should submit postmarketing adverse experience reports to:

Food and Drug Administration Center for Drug Evaluation and Research Central Document Room 5901-B Ammendale Road Beltsville, MD 20705-1266.

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at <a href="http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm">http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm</a>.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA 3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations sent by courier or overnight mail should be addressed to:

Food and Drug Administration Center for Drug Evaluation and Research Division of Compliance Risk Management and Surveillance 10903 New Hampshire Avenue, Bldg. 51, Room 4203 Silver Spring, MD 20992-0002

## CONTENT OF LABELING

FDA guidances at

Within 14 days of the date of this letter, submit content of labeling (21 CFR 601.14(b)) in structured product labeling (SPL) format, as described at

http://www.fda.gov/ForIndustrv/DataStandards/StructuredProductLabeling/default.htm that is identical in content to the enclosed labeling (text for the package insert and Medication Guide submitted November 27, 2009). The content of labeling should be submitted by updating your application by referencing the SPL file submitted to the drug establishment registration and drug listing system. To do this, place a link in your application submission that directs FDA to your SPL file. For administrative purposes, please designate this submission "Product Correspondence – Final SPL for approved BLA STN 125277." In addition, within 14 days of the date of this letter, amend any pending supplements for this BLA with content of labeling in SPL format to include the changes approved in this supplement. For additional information on submitting labeling to drug establishment registration and drug listing and to applications, see the

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072339.pdf and

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf,

## CARTON AND CONTAINER LABELS

Submit final printed carton and container labels that are identical to the labels submitted November 23, 2009, as soon as they are available but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005). Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "Product Correspondence – Final Printed Carton and Container Labels for approved BLA STN 125277." Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with labeling that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

### PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

You must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications, see <a href="http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm">http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm</a>.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

### LETTERS TO HEALTH CARE PROFESSIONALS

If you issue a letter communicating important safety-related information about this drug product (i.e., a "Dear Health Care Professional" letter), we request that you submit an electronic copy of the letter to both this BLA and to the following address:

MedWatch
Food and Drug Administration
Suite 12B-05
5600 Fishers Lane
Rockville, MD 20857

#### POST-ACTION FEEDBACK MEETING

New molecular entities and important new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during the drug development and marketing application review process. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, contact the Division of Pulmonary and Allergy Products.

If you have any questions, contact the Senior Regulatory Health Project Manager, Colette Jackson, at (301) 796-1230.

Sincerely,

/Curtis J. Rosebraugh, M.D., M.P.H./

Curtis J. Rosebraugh, M.D., M.P.H.

Director

Office of Drug Evaluation II

Center for Drug Evaluation and Research

Enclosures:

REMS documents
Package Insert
Medication Guide
Carton and Container Labels

# KALBITOR® BLA 125277

Dyax Corp. 300 Technology Square Cambridge, MA 02139

## RISK EVALUATION AND MITIGATION STRATEGY

#### I GOAL

To inform healthcare providers about the risk of anaphylaxis associated with KALBITOR and the importance of distinguishing between a hypersensitivity reaction and hereditary angioedema (HAE) attack symptoms.

To educate patients about the serious risks associated with KALBITOR therapy.

## II RISK EVALUATION AND MITIGATION STRATEGY ELEMENTS

#### A. Medication Guide

A Medication Guide will be dispensed with each KALBITOR dose. To ensure compliance with 21 CFR 208.24, a KALBITOR Medication Guide will be included in each dose unit of KALBITOR.

The KALBITOR carton packaging includes a prominent notice that each patient is required to receive the Medication Guide with each dose, as follows: "ATTENTION: Dispense the enclosed Medication Guide to each patient."

Please refer to the approved Medication Guide.

## B. Communication Plan

In accordance with FDCA 505-1(e)(3), a communication plan will be implemented by Dyax Corp. to convey important information about the risk of anaphylaxis and that the signs and symptoms of anaphylaxis and of acute attacks of HAE may overlap.

- The initial audience for this communication plan is healthcare providers likely to prescribe KALBITOR and treat HAE patients; including two specialties: Allergy/Immunology and Emergency Medicine.
- 2. The communication plan includes the Dear Healthcare Professional Letter that will describe the key risks of KALBITOR: [see attached DHCP Letter]

3. Distribution of materials: Communication plan materials will be distributed at the same time as product launch.

Direct Mail: Dyax will issue the DHCP Letter to targeted healthcare providers at the time of product launch and yearly for 2 years thereafter. In addition, for 2 years after launch, any known new prescribers of KALBITOR not previously targeted will also be sent the DHCP Letter. The DHCP Letter will include the warnings associated with KALBITOR and will describe symptoms of anaphylaxis that may overlap with presenting symptoms of an attack of HAE. The DHCP Letter will be sent by direct mail to providers in the specialties of Allergy/Immunology and Emergency Medicine. The DHCP Letter will include the Full Prescribing Information and the Medication Guide. Copies of these materials will also be available through a stand-alone webpage accessed through the product web site. (see attached webpage)

Dyax Representatives: The DHCP Letter will be provided with the Full Prescribing Information and Medication Guide by Dyax sales representatives to potential prescribers during the first discussion of KALBITOR during the first year of product availability.

The communication material listed in Section B.2 above will also be available at the time of approval by calling Dyax at 1-888-452-5248.

## C. Elements to Assure Safe Use

Elements to Assure Safe Use are not required.

## D. Implementation System

An Implementation System is not required.

# E. Timetable for Submission of Assessments

REMS assessments will be submitted at 18 months, 3 years and 7 years after approval. The reporting interval covered by each assessment will conclude no earlier than 60 days before the submission date for that assessment time interval. Each assessment will be submitted so that it is received by the FDA on or before the due date.

## [on Dyax letterhead]

#### IMPORTANT DRUG WARNING

#### Dear Healthcare Professional:

Dyax Corp. is writing to inform you of important safety information for KALBITOR® (ecallantide). KALBITOR is a subcutaneous injection indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age or older. Important safety information related to KALBITOR includes:

- The risk of anaphylaxis
- The need to distinguish signs and symptoms of anaphylaxis from HAE attacks

To ensure that the benefits of KALBITOR treatment outweigh the risks, the KALBITOR labeling includes a boxed warning concerning anaphylaxis, as follows:

WARNING: Anaphylaxis

Anaphylaxis has been reported after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR.

In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical trials, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. In clinical trials, when hypersensitivity was observed, it usually occurred immediately following exposure to KALBITOR, and always within the first hour following dosing.

In order to appropriately manage anaphylaxis, it must be recognized if it occurs. Because the signs and symptoms of HAE attacks may overlap with the signs and symptoms of anaphylaxis, there is a need to distinguish between serious hypersensitivity, including anaphylaxis and HAE attack symptoms.

Signs and symptoms that can be seen in either anaphylaxis or acute attacks of HAE include:

- erythema of the skin
- laryngeal edema
- dyspnea
- flushing
- stomach and gastrointestinal symptoms
- decreases in blood pressure

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. If a patient does not respond to an initial dose of KALBITOR, an additional dose of KALBITOR may be administered within a 24 hour period; before administering a repeat dose of KALBITOR, it is very important to assess the patient to assure that symptoms are reflective of an HAE attack and not a hypersensitivity reaction.

Please take time to read the enclosed KALBITOR Package Insert for full prescribing information.

In addition, please review the attached Medication Guide with each patient who is prescribed KALBITOR. The Medication Guide must be given to patients upon each use of KALBITOR and is supplied with each dose unit.

To report adverse events potentially associated with KALBITOR, please call Dyax Corp. at 1-888-452-5248. Alternatively, adverse event information may be reported to FDA's MedWatch Reporting System by:

- o Phone at 1-800-FDA-1088 (1-800-332-1088)
- o Facsimile at 1-800-FDA-0178 (1-800-332-0178)
- o Mail using FDA Form 3500 located at http://www.fda.gov/medwatch

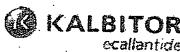
We invite you to contact Dyax Corp. at 1-888-452-5248 if you have any questions about KALBITOR or the information in this letter.

Sincerely,

Patrick T. Horn, MD, PhD Vice President, Clinical and Medical Affairs Dyax Corp.

Enclosures: Full Prescribing Information and Medication Guide





# IMPORTANT SAFETY INFORMATION FOR HEALTHCARE PROFESSIONALS

## Risk Evaluation and Mitigation Strategy (REMS)

A Risk Evaluation and Mitigation Strategy (REMS) is a strategy to manage known or potential serious risks associated with a drug product and is required by the Food and Drug Administration to ensure that the benefits of the drug outweigh its risks.

In order for Dyax to communicate certain risks to ensure that KALBITOR is prescribed and taken safely, Dyax has worked with the FDA to develop materials to communicate the risk of anaphylaxis and the importance of distinguishing between hypersensitivity reactions and ongoing hereditary angioedema (HAE) symptoms. The REMS program is designed to inform healthcare providers and patients about the potential risks with KALBITOR. To learn more about serious risks, read the important safety information provided in this link, including the Medication Guide, and discuss it with your patients.

The goals of the KALBITOR REMS are:

- To inform healthcare providers about the risk of anaphylaxis associated with KALBITOR and the importance of distinguishing between a
  hypersensitivity reaction and hereditary angloedema (HAE) attack symptoms.
- . To educate patients about the serious risks associated with KAI BITOR therapy.

To download the REMS documents:

- Dear Healthcare Professional Letter odf

- Prescribing Information.pdf

Medication Guide, adf

#### Important Safety Information

#### WARNING: Anaphylaxis

Anaphylaxis has been reported after administration of KALBITOR®. Because of the risk of anaphylaxis, KALBITOR should only be administered by a realthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity.

#### CONTRAINDICATIONS

Do not administer KALDITOR to a patient who has known clinical hypersensitivity to KALBITOR

#### WARNINGS AND PRECAUTIONS

In 255 HAE patients freated with intravenous or subcutaneous KALBITOR in clinical trials, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. These reactions occurred within the first hour after nosing

Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruntus, inhorrhea, sneezing, nasal congestion, throat irritation, unticana, wheezing, and hypotens-on

Patients should be observed for an appropriate period of time after administration of KALBITOR, raking into account the time to onset of anaphylaxis seen in clinical trials. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

#### **ADVERSE EVENTS**

The most common adverse events (≥3% and greater than placebo) in HAR patients were headache, nauses, diarrhea, byrexia, injection site reactions, and nasopharyneitis. There is a potential for immunogenicity with the use of KALSITOR, Patients who peroconvert may be at a higher risk of a hypersensitivity reaction. The long-term effects of antibodies to KALSITOR are not known.

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Please see the [4] Prescripte information including Boxed Warning and Medication Guido.

Healthcare professionals should report all suspected adverse events associated with the use of KALBITOR. Please centact Dyax Corp. at 1-888-452-5248. Alternatively, this information may be reported to the FDA MedWatch System by phone at 1-800-FDA-1088 or by mail using Form 3500 at www.fda.gov/medwatch.

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Dyax Corp.

HIGHLIGHTS OF PRESCRIBING INFORMATION These highlights do not include all the information needed to use KALBITOR® safely and effectively. See full prescribing information for KALBITOR.

KALBITOR (ecallantide) injection, for subcutaneous use Initial U.S. Approval: [year]

#### WARNING-Anaphylaxis

(Sec full prescribing information for complete boxed warning)

Anaphylaxis has been reported after administration of KALBITOR<sup>®</sup>. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)].

INDICATIONS AND USAGE-

KALBITOR is a plasma kallikrein inhibitor indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of

DOSAGE AND ADMINISTRATION-----

- 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If an attack persists, an additional dose of 30 mg may be administered within a 24 hour period. (2.1)
  - KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. (2.2).

-DOSAGE FORMS AND STRENGTHS-

Single use glass vial containing 10 mg/mL of ecallantide as a solution for injection. (3)

-CONTRAINDICATIONS-

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. (4)

-WARNINGS AND PRECAUTIONS-

- Hypersensitivity Reactions Including Anaphylaxis: Anaphylaxis has occurred in 3.9% of treated patients. Administer KALBITOR in a setting equipped to manage anaphylaxis and hereditary angioedema. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, monitor patients closely for hypersensitivity reactions (5).
- -ADVERSE REACTIONS-The most common adverse reactions occurring in ≥3% of KALBITORtreated patients and greater than placebo are headache, nausea, diarrhea, pyrexia, injection site reactions, and nasopharyngitis. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Dyax Corp. at 1-888-452-5248 or FDA at 1-800-FDA-1088 or www.fda.gow/medwatch

See 17 for PATIENT COUNSELING INFORMATION and Medication

Revised: [m/year]

#### FULL PRESCRIBING INFORMATION: CONTENTS\* WARNING: ANAPHYLAXIS

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<sup>\*</sup>Sections or subsections omitted from the full prescribing information are not listed.

## **FULL PRESCRIBING INFORMATION**

## WARNING: Anaphylaxis

Anaphylaxis has been reported after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR. [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)]

## 1 INDICATIONS AND USAGE

KALBITOR® (ecallantide) is indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older.

## 2 DOSAGE AND ADMINISTRATION

## 2.1 Recommended Dosing

The recommended dose of KALBITOR is 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If the attack persists, an additional dose of 30 mg may be administered within a 24 hour period.

## 2.2 Administration Instructions

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema.

KALBITOR should be refrigerated and protected from the light. KALBITOR is a clear, colorless liquid; visually inspect each vial for particulate matter and discoloration prior to administration. If there is particulate matter or discoloration, the vial should not be used.

Using aseptic technique, withdraw 1 mL (10 mg) of KALBITOR from the vial using a large bore needle. Change the needle on the syringe to a needle suitable for subcutaneous injection. The recommended needle size is 27 gauge. Inject KALBITOR into the skin of the abdomen, thigh, or upper arm. Repeat the procedure for each of the 3 vials comprising the KALBITOR dose. The injection site for each of the injections may be in the same or in different anatomic locations (abdomen, thigh, upper arm). There is no need for site rotation. Injection sites should be separated by at least 2 inches (5 cm) and away from the anatomical site of attack.

The same instructions apply to an additional dose administered within 24 hours. Different injection sites or the same anatomical location (as used for the first administration) may be used.

# 3 DOSAGE FORMS AND STRENGTHS

KALBITOR is a clear, colorless liquid free of preservatives. Each vial of KALBITOR contains ecallantide at a concentration of 10 mg/mL.

## 4 CONTRAINDICATIONS

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. [see Warnings and Precautions (5.1)].

# 5 WARNINGS AND PRECAUTIONS

# 5.1 Hypersensitivity Reactions, Including Anaphylaxis

Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR. In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical studies, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruritus, rhinorrhea, sneezing, nasal congestion, throat irritation, urticaria, wheezing, and hypotension. These reactions occurred within the first hour after dosing.

Other adverse reactions indicative of hypersensitivity reactions included the following: pruritus (5.1%), rash (3.1%), and urticaria (2.0%).

Patients should be observed for an appropriate period of time after administration of KALBITOR, taking into account the time to onset of anaphylaxis seen in clinical trials. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

KALBITOR should not be administered to any patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4)].

# **6 ADVERSE REACTIONS**

Hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR [see Contraindications (4) and Warnings and Precautions (5.1)].

# 6.1 Clinical Trials Experience

Because clinical trials are conducted under varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety data described below reflect exposure to KALBITOR in 255 patients with HAE treated with either intravenous or subcutaneous KALBITOR. Of the 255 patients,

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66% of patients were female and 86% were Caucasian. Patients treated with KALBITOR were between the ages of 10 and 78 years.

Overall, the most common adverse reactions in 255 patients with HAE were headache (16.1%), nausea (12.9%), fatigue (11.8%), diarrhea (10.6%), upper respiratory tract infection (8.2%), injection site reactions (7.4%), nasopharyngitis (5.9%), vomiting (5.5%), pruritus (5.1%), upper abdominal pain (5.1%), and pyrexia (4.7%). Anaphylaxis was reported in 3.9% of patients with HAE. Injection site reactions were characterized by local pruritus, erythema, pain, irritation, urticaria, and/or bruising.

The incidence of adverse reactions below is based upon 2 placebo-controlled, clinical trials (EDEMA3® and EDEMA4®) in a total of 143 unique patients with HAE. Patients were treated with KALBITOR 30 mg subcutaneous or placebo. Patients were permitted to participate sequentially in both placebo-controlled trials; safety data collected during exposure to KALBITOR was attributed to treatment with KALBITOR, and safety data collected during exposure to placebo was attributed to treatment with placebo. Table 1 shows adverse reactions occurring in  $\geq 3\%$  of KALBITOR-treated patients that also occurred at a higher rate than in the placebo-treated patients in the two controlled trials (EDEMA3 and EDEMA4) of the 30 mg subcutaneous dose.

Table 1: Adverse Reactions Occurring at ≥3% and Higher than Placebo in 2 Placebo Controlled Clinical Trials in Patients with HAE Treated with KALBITOR

	KALBITOR N=100	Placebo N=81
Adverse Reactions	n (%) ª	n (%)*
Headache	8 (8%)	6 (7%)
Nausea	5 (5%)	1 (1%)
Diarrhea	4 (4%)	3 (4%)
Pyrexia	4 (4%)	0
Injection site reactions	3 (3%)	1 (1%)
Nasopharyngitis	3 (3%)	0

Patients experiencing more than 1 event with the same preferred term are counted only once for that preferred term.

Some patients in EDEMA3 and EDEMA4 received a second, open-label 30 mg subcutaneous dose of KALBITOR within 24 hours following the initial dose. Adverse reactions reported by these patients who received the additional 30 mg subcutaneous dose of KALBITOR were consistent with those reported in the patients receiving a single dose.

# 6.2 Immunogenicity

In the KALBITOR HAE program, patients developed antibodies to KALBITOR. Rates of seroconversion increased with exposure to KALBITOR over time. Overall, 7.4% of patients seroconverted to anti-ecallantide antibodies. Neutralizing antibodies to ecallantide were determined *in vitro* to be present in 4.7% of patients.

Anti-ecallantide and anti-P. pastoris IgE antibodies were also detected. Patients who seroconvert may be at a higher risk of a hypersensitivity reaction. The long-term effects of antibodies to KALBITOR are not known.

The test results for the ecallantide program were determined using one of two assay formats: ELISA and bridging electrochemiluminescence (ECL). As with all therapeutic proteins, there is a potential for immunogenicity with the use of KALBITOR. The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to KALBITOR with the incidence of antibodies to other products may be misleading.

## 7 DRUG INTERACTIONS

No formal drug interactions studies were performed. No *in vitro* metabolism studies were performed.

## 8 USE IN SPECIFIC POPULATIONS

## 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled trials of KALBITOR in pregnant women. KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Because animal reproductive studies are not always predictive of human response, KALBITOR should be used during pregnancy only if clearly needed.

In rats, intravenous KALBITOR at an intravenous dose approximately 13 times the maximum recommended human dose (MRHD) on a mg/kg basis caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. No development toxicity was observed in rats that received an intravenous dose approximately 8 times the MRHD on a mg/kg basis. There were no adverse effects of KALBITOR on embryofetal development in rats that received subcutaneous doses up to approximately 2.4 times the MRHD on an AUC basis, and in rabbits that received intravenous doses up to approximately 6 times the MRHD on an AUC basis.

## 8.2 Labor and Delivery

No information is available on the effects of KALBITOR during labor and delivery.

## 8.3 Nursing Mothers

It is not known whether ecallantide is excreted in human milk. Caution should be exercised when ecallantide is administered to a nursing woman.

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### 8.4 Pediatric Use

Safety and effectiveness of KALBITOR in patients below 16 years of age have not been established.

### 8.5 Geriatric Use

Clinical trials of KALBITOR did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

### 10 OVERDOSAGE

There have been no reports of overdose with KALBITOR. HAE patients have received single doses up to 90 mg intravenously without evidence of dose-related toxicity. No deaths occurred in monkeys that received intravenous or subcutaneous doses up to 25 mg/kg (approximately 22 times the MRHD on an AUC basis).

### 11 DESCRIPTION

KALBITOR (ecallantide) is a human plasma kallikrein inhibitor for injection for subcutaneous use.

KALBITOR is a clear and colorless, sterile, and nonpyrogenic solution. Each vial contains 10 mg ecallantide as the active ingredient, and the following inactive ingredients: 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, and 8 mg sodium chloride in water for injection, USP. KALBITOR is preservative free, with a pH of approximately 7.0. A 30 mg dose is supplied as 3 vials each containing 1 mL of 10 mg/mL KALBITOR. Each vial contains a slight overfill. Vials are intended for single use. Ecallantide is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology.

### 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on Chromosome 11q and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kininogen to bradykinin by the protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During

attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Bradykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein. KALBITOR binds to plasma kallikrein and blocks its binding site, inhibiting the conversion of HMW kininogen to bradykinin. By directly inhibiting plasma kallikrein, KALBITOR reduces the conversion of HMW kininogen to bradykinin and thereby treats symptoms of the disease during acute episodic attacks of HAE.

### 12.2 Pharmacodynamics

No exposure-response relationships for KALBITOR to components of the complement or kallikrein-kinin pathways have been established.

The effect of KALBITOR on activated partial thromboplastin time (aPTT) was measured because of potential effect on the intrinsic coagulation pathway. Prolongation of aPTT has been observed following intravenous dosing of KALBITOR at doses ≥20 mg/m². At 80 mg administered intravenously in healthy subjects, aPTT values were prolonged approximately two-fold over baseline values and returned to normal by 4 hours post-dose.

For patients taking KALBITOR, no significant QT prolongation has been seen. In a randomized, placebo-controlled trial (EDEMA4) studying the 30 mg subcutaneous dose versus placebo, 12-lead ECGs were obtained at baseline, 2 hours and 4 hours post-dose (covering the time of expected  $C_{max}$ ), and at follow-up (day 7). ECGs were evaluated for PR interval, QRS complex, and QTc interval. KALBITOR had no significant effect on the QTc interval, heart rate, or any other components of the ECG.

### 12.3 Pharmacokinetics

Following the administration of a single 30 mg subcutaneous dose of KALBITOR to healthy subjects, a mean ( $\pm$  standard deviation) maximum plasma concentration of  $586 \pm 106$  ng/mL was observed approximately 2 to 3 hours post-dose. The mean area under the concentration-time curve was  $3017 \pm 402$  ng\*hr/mL. Following administration, plasma concentration declined with a mean elimination half-life of  $2.0 \pm 0.5$  hours. Plasma clearance was  $153 \pm 20$  mL/min and the volume of distribution was  $26.4 \pm 7.8$  L. Based on a population pharmacokinetic analysis, body weight, age, and gender were not found to affect KALBITOR exposure significantly. Ecallantide is a small protein (7054 Da) and renal elimination in the urine of treated subjects has been demonstrated.

No pharmacokinetic data are available in patients or subjects with hepatic or renal impairment.

## 13 NONCLINICAL TOXICOLOGY

## 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There are no animal or human studies to assess the carcinogenic or mutagenic potential of KALBITOR (ecallantide).

KALBITOR had no effects on fertility and reproductive performance in rats at subcutaneous doses up to 25 mg/kg/day (approximately 21 times the MRHD on a mg/kg basis).

### 13.2 Animal Toxicology

Reproductive Toxicology Studies

KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Treatment of rats with an intravenous dose of 15 mg/kg/day (approximately 13 times the MRHD on a mg/kg basis) caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. However, no development toxicity was observed in rats that received an intravenous dose of 10 mg/kg/day (approximately 8 times the MRHD on a mg/kg basis). KALBITOR was not teratogenic in rats at subcutaneous doses up to 20 mg/kg/day (approximately 2.4 times the MRHD on an AUC basis) and rabbits that received intravenous doses up to 5 mg/kg/day (approximately 6 times the MRHD on an AUC basis).

### 14 CLINICAL STUDIES

The safety and efficacy of KALBITOR was evaluated in 2 randomized, double-blind, placebo-controlled trials (EDEMA4 and EDEMA3) in 168 patients with HAE. Patients having an attack of hereditary angioedema, at any anatomic location, with at least 1 moderate or severe symptom, were treated with 30 mg subcutaneous KALBITOR or placebo. Because patients could participate in both trials, a total of 143 unique patients participated. Of the 143 patients, 94 were female, 123 were Caucasian, and the mean age was 36 years. There were 64 patients with abdominal attacks, 55 with peripheral attacks, and 24 with laryngeal attacks.

In both trials, the effects of KALBITOR were evaluated using the Mean Symptom Complex Severity (MSCS) score and the Treatment Outcome Score (TOS). These measures evaluated the severity of attack symptoms at all anatomical locations (MSCS score) and response to therapy (TOS).

MSCS score is a point-in-time measure of symptom severity. At baseline, 4 hours, and 24 hours, patients rated the severity on a categorical scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for symptoms at each affected anatomical location. Ratings were averaged to obtain the MSCS score. A decrease in MSCS score reflected an improvement in symptoms.

TOS is a measure of symptom response to treatment. At 4 hours and 24 hours, patient assessment of response characterized by their change from baseline in symptom severity and collected by anatomic site of attack involvement, was recorded on a categorical scale (significant improvement [100], improvement [50], same [0], worsening [-50], significant worsening [-100]). The response at each anatomic site was weighted by baseline severity and then the weighted scores across all involved sites were averaged to calculate the TOS. A TOS value >0 reflected an improvement in symptoms from baseline.

### **EDEMA4**

EDEMA4 was a randomized, double-blind, placebo-controlled trial in which 96 patients were randomized 1:1 to receive KALBITOR 30 mg subcutaneous or placebo for acute attacks of HAE. The primary endpoint was the change from baseline in MSCS score at 4 hours, and the TOS at 4 hours was a key secondary endpoint. Patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients with placebo and the results were statistically significant (Table 2). At 24 hours, patients treated with KALBITOR also demonstrated a greater decrease from baseline in the MSCS than placebo (-1.5 vs. -1.1; p = 0.04) and a greater TOS (89 vs. 55, p = 0.03).

Table 2: Change in MSCS Score and TOS at 4 Hours

•	EDE	MA4	EDE!	MA3
	KALBITOR	Placebo	KALBITOR	Placebo
<del></del>	(N=48)	(N=48)	(N=36)	(N=36)
Change in MS	CS Score at 4 Hours			•
n	47	42	34	35
Mean	-0.8	-0.4	-1.1	-0.6
95% CI	-1.0, -0.6	-0.6, -0.1	-1.4, -0.8,	-0.8, -0.4
P-value	0.0	010	0.04	11
TOS at 4 Hour	<u>'s</u>			
D	47	42	34	35
Mean	53	8	63	36
95% CI	39, 68	-12, 28	49, 76	17, 54
P-value	0.0	03	0.04	

MSCS: Mean Symptom Complex Severity

TOS: Treatment Outcome Score

CI: confidence interval

More patients in the placebo group (24/48, 50%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (16/48, 33%).

Some patients reported improvement following a second 30 mg subcutaneous dose of KALBITOR, administered within 24 hours following the initial dose for symptom persistence or relapse, but efficacy was not systematically assessed for the second dose.

### **EDEMA3**

EDEMA3 was a randomized, double-blind, placebo-controlled trial in which 72 patients were randomized 1:1 to receive KALBITOR or placebo for acute attacks of HAE. EDEMA3 was similar in design to EDEMA4 with the exception of the order of the prespecified efficacy endpoints. In EDEMA3, the primary endpoint was the TOS at 4 hours, and the key secondary efficacy endpoint was the change from baseline in MSCS at 4 hours. As in EDEMA4, patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients treated with placebo and the results were statistically significant (Table 2).

In addition, more patients in the placebo group (13/36, 36%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (5/36, 14%).

### 16 HOW SUPPLIED/STORAGE AND HANDLING

KALBITOR (ecallantide) is supplied as three 10 mg/mL single-use vials packaged in a carton. Each vial contains 10 mg of ecallantide. Each vial contains a slight overfill.

NDC (47783-101-01): 3 single-use vials in 1 carton

KALBITOR should be kept refrigerated (2°C to 8°C/36°F to 46°F). Vials removed from refrigeration should be stored below 86°F/30°C and used within 14 days or returned to refrigeration until use.

Protect vials from light until use.

Do not use beyond the expiration date.

Draft November 27, 2009

### 17 PATIENT COUNSELING INFORMATION

- Patients should be advised that KALBITOR may cause anaphylaxis and other
  hypersensitivity reactions. Patients should be advised that KALBITOR should be
  administered by a healthcare professional with appropriate medical support to
  manage anaphylaxis and hereditary angioedema. Patients who have known
  clinical hypersensitivity to KALBITOR should be instructed not to receive
  additional doses of KALBITOR. [see Boxed Warning, Contraindications (4), and
  Warnings and Precautions (5.1)]
- Patients should be advised to consult the Medication Guide for additional information regarding the risk of anaphylaxis and other hypersensitivity reactions.

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### **Medication Guide**

### KALBITOR® (KAL-bi-tor)

### (ecallantide)

Read this Medication Guide before you start receiving KALBITOR and before each treatment. There may be new information. This Medication Guide does not take the place of talking to your doctor about your medical condition or your treatment.

### What is the most important information that I should know about KALBITOR?

Serious allergic reactions may happen in some people who receive KALBITOR. These allergic reactions can be life-threatening and usually happen within 1 hour after receiving KALBITOR.

- KALBITOR should be given to you by a doctor or nurse in a healthcare setting where serious allergic reactions and hereditary angioedema (HAE) can be treated.
- Symptoms of a serious allergic reaction to KALBITOR can be similar to the symptoms of HAE, the condition that you are being treated for. Your doctor or nurse should watch you for any signs of a serious allergic reaction after treatment with KALBITOR.
- Tell your doctor or nurse right away if you have any of these symptoms of a serious allergic reaction during or after treatment with KALBITOR:
  - wheezing, shortness of breath, cough, chest tightness, or trouble breathing
  - dizziness, fainting, fast or weak heartbeat, or feeling nervous
  - reddening of the face, itching, hives, or feeling warm
  - swelling of the throat or tongue, throat tightness, hoarse voice, or trouble swallowing
  - runny nose or sneezing

### What is KALBITOR?

KALBITOR is a prescription medicine used to treat sudden attacks of hereditary angioedema (HAE).

KALBITOR is not a cure for HAE.

It is not known if KALBITOR is safe and effective in children under 16 years of age.

### Who should not receive KALBITOR?

Do not receive KALBITOR if you are allergic to KALBITOR.

### What should I tell my doctor before I receive KALBITOR?

Before receiving KALBITOR, tell your doctor if you:

- have ever had an allergic reaction to KALBITOR. See "Who should not take KALBITOR?"
- are pregnant or plan to become pregnant. It is not known if KALBITOR will harm your unborn baby.
- are breast-feeding or plan to breast-feed. It is not known if KALBITOR passes into your breast milk.

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Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list of them to show to your doctor and pharmacist when you get a new medicine.

### How will I receive KALBITOR?

For each dose, you will receive 3 injections just under the skin (subcutaneous or SC injections) of your abdomen, thigh, or upper arm.

### What are the possible side effects?

KALBITOR can cause serious allergic reactions. See "What is the most important information I should know about KALBITOR?").

Common side effects of KALBITOR include:

- headache
- nausea
- diarrhea
- fever
- · injection site reactions, such as redness, rash, swelling, itching, or bruising
- stuffy nose

Call your doctor for advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

### General information about KALBITOR

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. This Medication Guide gives you the most important information about KALBITOR. If you would like more information, talk with your doctor. You can ask your pharmacist or doctor for information about KALBITOR that is written for health professionals.

### What are the ingredients of KALBITOR?

Active Ingredient: ecallantide

Inactive ingredients: disodium hydrogen orthophosphate (dihydrate), monopotassium phosphate, potassium chloride, sodium chloride in water for injection.

Manufactured for: Dyax Corp.

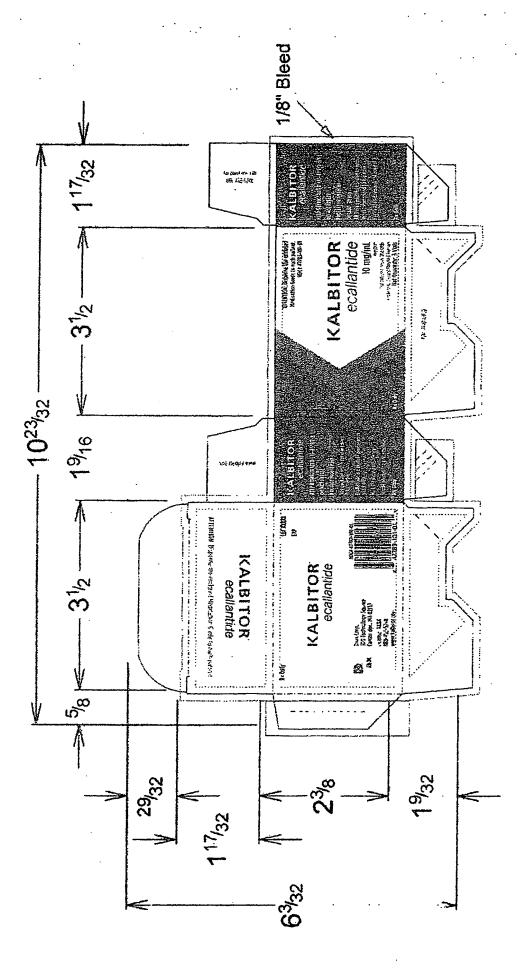
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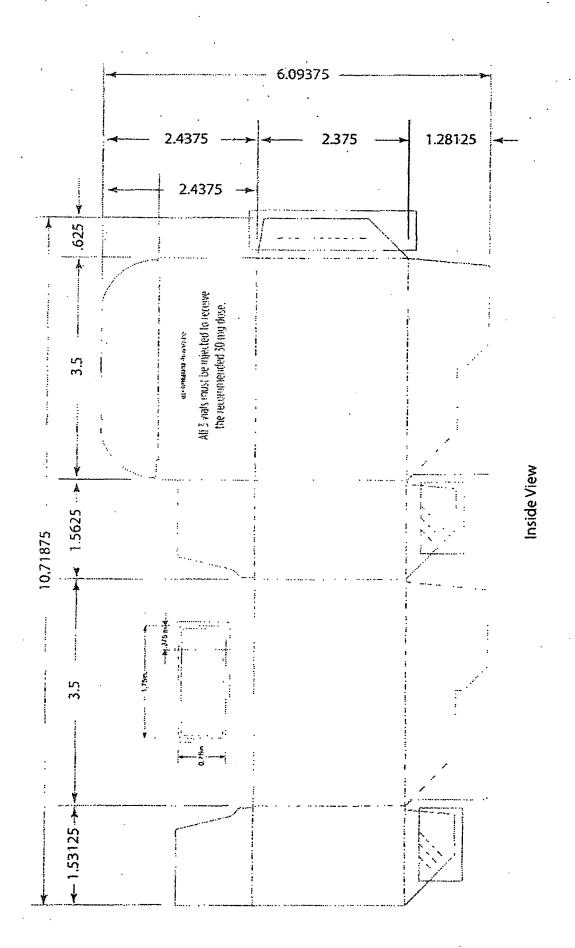
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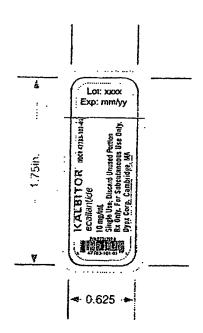
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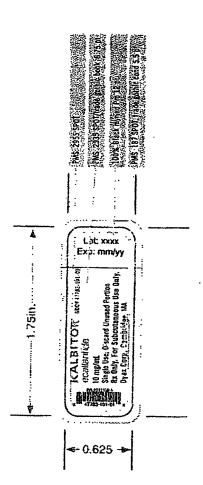
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Lot: xxxx Exp: mm/yy

10 mg/mL Single Use; Discard Unused Portion Rx Only. For Subcutaneous Use Only. Dyax Corp. Cambridge, MA NDC# 47783-101-01 ecallantide

TO L BUS

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

Attachment E

U.S. Patent No. 5,795,865

### ATTACHMENT E

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### [54] KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND ANALOGUES THEREOF

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514/12; 435/4, 7.1, 7.6, 7.71, 7.72

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#### [57] **ABSTRACT**

Proteins are disclosed that are homologous to bovine pancreatic trypsin inhibitor (BPTI) Kunitz domains, and especially proteins that are homologous to lipoprotein-associated coagulation inhibitor (LACI) Kunitz domains, which inhibit one or more plasma and/or tissue kallikreins, and uses of such proteins in therapeutic and diagnostic methods also are disclosed. In particular, Kunitz domains derived from Kunitz domains of human origin and especially to the first Kunitz domain of LACI are disclosed.

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THEREOF

### KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND ANALOGUES

This application is a 371 of PCT/US95/00299 which is 5 continuation-in-part of U.S. application Ser. No. 08/208, 264, filed Mar. 10, 1994 now abandoned, and is a continuation-in-part of application Ser. No. 08/179,904. filed Jan. 11, 1994, now abandoned, both of which application are hereby incorporated by reference in their entireties. 10

### BACKGROUND OF THE INVENTION

### 1. Field of the Invention

This invention relates to novel classes of proteins and protein analogues which bind to and inhibit human plasma

### 2. Description of the Background Art

Kallikreins are serine proteases found in both tissues and plasma. Plasma kallikrein is involved in contact-activated 20 (intrinsic pathway) coagulation, fibrinolysis, hypotension, and inflammation. (See BHOO92). These effects of kallikrein are mediated through the activities of three distinct physiological substrates: i) Factor XII (coagulation). ii) gens (hypotension and inflammation).

Kallikrein cleavage of kininogens results in the production of kinins, small highly potent bioactive peptides. The kinins act through cell surface receptors present on a variety of cell types. Intracellular heterotrimeric G-proteins link the kinin receptors to second messenger pathways including nitric oxide, adenyl cyclase, phospholipase A2, and phospholipase C. Among the significant physiological activities of kinins are: (i) increased vascular permeability; (ii) vasodilation; (iii) bronchospasm; and (iv) pain induction. Thus. 35 kinins mediate the life-threatening vascular shock and edema associated with bacteremia (sepsis) or trauma, the edema and airway hyperreactivity of asthma, and both inflammatory and neurogenic pain associated with tissue injury. The consequences of inappropriate plasma kallikrein activity and resultant kinin production are dramatically illustrated in patients with hereditary angioedema (HA). HA is due to a genetic deficiency of C1-inhibitor, the principal endogenous inhibitor of plasma kallikrein. Symptoms of HA include edema of the skin, subcutaneous tissues and gastrointestinal tract, and abdominal pain and vomiting. Nearly one-third of HA patients die by suffocation due to edema of the larynx and upper respiratory tract. Kallikrein is secreted as a zymogen (prekallikrein) that circulates as an inactive molecule until activated by a proteolytic event that frees the +NH3-IVGGTNSS ... sequence of kallikrein (SEQ ID NO. 1). Human Plasma Prekallikrein is found in Genebank entry

Mature plasma Kallikrein contains 619 amino acids. Hydrolysis of the Arg<sub>371</sub>-Ile<sub>372</sub> peptide bond yields a twochain proteinase joined by a disulfide bond. The aminoterminal light chain (248 residues) carries the catalytic site.

The main inhibitor of plasma kallikrein (pKA) in vivo is the C1 inhibitor; see SCHM87, pp.27-28. C1 is a serpin and forms an essentially irreversible complex with pKA. 60 Although bovine pancreatic trypsin inhibitor (BPTI) was first said to be a strong pKA inhibitor with K<sub>1</sub>=320 pM (AUER88). BERN93 indicates that its K, for pKA is 30 nM (i.e., 30,000 pM). The G36S mutant had a  $K_i$  of over 500 essential attributes of such an agent are:

i. Neutralization of relevant kallikrein enzyme(s);

- ii. High affinity binding to target kallikreins to minimize dose:
- iii. High specificity for kallikrein, to reduce side effects; and
- iv. High degree of similarity to a human protein to minimize potential immunogenicity and organ/tissue toxicity.

The candidate target kallikreins to be inhibited are chymotrypsin-homologous serine proteases.

### **Excessive Bleeding**

Excessive bleeding can result from deficient coagulation activity, elevated fibrinolytic activity, or a combination of the two. In most diatheses one must controll the activity of plasmin. However, plasma kallikrein (pKA) is an activator of plasminogen and a potent, selective pKA inhibitor may avert plasminogen activation. The clinically beneficial effect of BPTI in reducing blood loss is thought to result from its inhibition of plasmin ( $K_D$ -0.3 nM) or of plasma kallikrein  $(K_D-100 \text{ nM})$  or both enzymes. It has been found, however, that BPTI is sufficiently antigenic that second uses require skin testing. Furthermore, the doses of BPTI required to control bleeding are quite high and the mechanism of action is not clear. Some say that BPTI acts on plasmin while others Pro-urokinase/plasminogen (fibrinolysis), and iii) Kinino- 25 say that it acts by inhibiting plasma kallikrein. FRAE89 reports that doses of about 840 mg of BPTI to 80 open-heart surgery patients reduced blood loss by almost half and the mean amount transfused was decreased by 74%. Miles Inc. has recently introduced Trasylol in the USA for reduction of bleeding in surgery (See Miles product brochure on Trasylol. which is hereby incorporated by reference.) LOHM93 suggests that plasmin inhibitors may be useful in controlling bleeding in surgery of the eye. SHER89 reports that BPTI may be useful in limiting bleeding in colonic surgery.

A kallikrein inhibitor that is much more potent than BPTI and that is almost identical to a human protein domain offers similar therapeutic potential, allows dose to be reduced, and poses less potential for antigenicity.

With recombinant DNA techniques, one may obtain a novel protein by expression of a mutated gene of a parental protein. Several strategies are known for picking mutations to test. One, "protein surgery", involves the introduction of one or more predetermined mutations within the gene of choice. A single polypeptide of completely predetermined 45 sequence is expressed, and its binding characteristics are

At the other extreme is random mutagenesis by means of relatively nonspecific mutagens such as radiation and various chemical agents, see Lehtovaara, E.P. Appln. 285,123. or by expression of highly degenerate DNA. It is also possible to follow an intermediate strategy in which some residues are kept constant, others are randomly mutated, and still others are mutated in a predetermined manner. This is called "variegation". See Ladner, et al. U.S. Pat. No. 5.220. 55 409.

DENN94a and DENN94b report selections of Kunitz domains based on APP-I for binding to the complex of Tissue Factor with Factor VII. They did not use LACI-K1 as parental and did not use pKA as a target. The highest affinity binder they obtained had  $K_D$  for their target of about 2 nM. Our first-round selectants for binding to pKA have affinity of about 0.3 nM, and our second round selectants are about at 0.1 nM (=100 pM) or better.

nM. Thus, there is a need for a safe kallikrein inhibitor. The 65 less likely to cause an immune response when injected into Proteins taken from a. particular species are assumed to be individuals of that species. Murine antibodies are highly antigenic in humans. "Chimeric" antibodies having human

constant domains and murine variable domains are decidedly less antigenic. So called "humanized" antibodies have human constant domains and variable domains in which the CDRs are taken from murine antibodies while the framework of the variable domains are of human origin. "Humanized" antibodies are much less antigenic than are "chimeric" antibodies. In a "humanized" antibody, fifty to sixty residues of the protein are of non-human origin. The proteins of this invention comprise, in most cases, only about sixty amino acids and usually there are ten or fewer differences between the engineered protein and the parental protein. Although humans do develop antibodies even to human proteins, such as human insulin, such antibodies tend to bind weakly and the often do not prevent the injected protein from displaying its intended biological function. Using a protein from the species to be treated does not guarantee that there will be no immune response. Nevertheless, picking a protein very close in sequence to a human protein greatly reduces the risk of strong immune response in humans.

Kunitz domains are highly stable and can be produced 20 efficiently in yeast or other host organisms. At least ten human Kunitz domains have been reported. Although BPTI was thought at one time to be a potent pKA inhibitor, there are, actually, no human Kunitz domains that inhibits pKA very well. Thus, it is a goal of this invention to provide sequences of Kunitz domain that are both potent inhibitors of pKA and close in sequence to human Kunitz domains.

The use of site-specific mutagenesis, whether nonrandom or random, to obtain mutant binding proteins of improved activity, is known in the art, but does not guarantee that the mutant proteins will have the desired target specificity or affinity. Given the poor anti-kallikrein activity of BPTI, mutation of BPTI or other Kunitz domain proteins would not have been considered, prior to this invention, a preferred method of obtaining a strong binder, let alone inhibitor, of 35 kallikrein.

### SUMMARY OF THE INVENTION

This invention relates to novel BPTI-homologous Kunitz domains, especially LACI homologues, which inhibit one or more plasma (and/or tissue) kallikreins, and to the thera- 40 peutic and diagnostic use of these novel proteins. In particular, this invention relates to Kunitz domains derived from Kunitz domains of human origin and especially to the first Kunitz domain of LACI; Kunitz domains of human proteins of this invention inhibit plasma kallikrein (and/or tissue kallikrein) with a  $K_D$  of no more than 20 nM. preferably, no more than 5 nM, more preferably, no more than about 300 pM, and most preferably, no more than about 100 pM.

A specific, high affinity inhibitor of plasma kallikrein (and, where needed, tissue kallikrein) will demonstrate significant therapeutic utility in all pathological conditions mediated by kallikrein, and especially those associated with kinins. The therapeutic approach of inhibiting the catalytic 55 production of kinins is considered preferable to antagonism of kinin receptors, since in the absence of kallikrein inhibition, receptor antagonists must compete with continuous kinin generation. Significantly, genetic deficiency of kallikrein is likely to be safe. We have recently discovered a lead pKA inhibitor, designated KKII/3#6. This inhibitor is a variant of a naturally occurring human plasma protein Kunitz domain and demonstrates significantly greater kallikrein binding potency than Trasylol. KKII/3#6 has a K. for 65 kallikrein which is over 100 times that of both wild-type LACI and of BPII, and is about 300 pM. In contrast, its K.

for plasmin is 10 µM. Proteins KK2/#11 and KK2/#13 are especially preferred pKA inhibitors and have K, <300 pM and probably less than 100 pM. A reversible inhibitor is believed to be of greater utility than an irreversible inhibitor 5 such as the C1 inhibitor.

Transfer of the subsequences that confer pKA binding into other Kunitz domains, particularly human Kunitz domains is

The preferred pKA inhibitors of the present invention 10 fullfil one or more of the following desiderata:

- 1) the inhibitor inhibits plasma kallikrein with a Ki no more than 20 nM. preferably 5 nM or less, more preferably 300 pM or less, and most preferably 100 pM
- 2) the inhibitor comprises a Kunitz domain meeting the requirements shown in Table 14 with residues numbered by reference to BPTI,
- 3) the inhibitor has at the Kunitz domain positions 12-21 and 32-39 one of the amino-acid types listed for that position in Table 15, and
- 4) the inhibitor is substantially homologous to a reference sequence of essentially human origin selected from the group KKII/3#6, KK2/#11, KK2/#13, KK2/#1, KK2/ #2, KK2/#3, KK2/#4, KK2/#6, KK2/#7, KK2/#8, KK2/#9, KK2/#10, KK2/#12, KK2conl, Human LACI-K2, Human LACI-K3, Human collagen µ3 KuDom, Human TFPI-2 DOMAIN 1. Human TFPI-2 DOMAIN 2. Human TFPI-2 DOMAIN 3. HUMAN ITI-K1. Human ITI-K2. HUMAN PROTEASE NEXIN-II. Human APP-I, DKI-1.2.1, DKI-1.3.1, DKI-2.1, DKI-3.1.1. DKI-3.2.1. DKI-3.3.1. DKI-4.1.1. DKI-4.2.1. DKI-4.2.2, DKI-5.1, and DKI-6.1

#### Nomenclature

Herein, affinities are stated as  $K_D(K_D(A,B)=[A][B]/[A-B]$ ). A numerically smaller  $K_D$  reflects higher affinity. For the purposes of this invention, a "kallikrein inhibiting protein" is one that binds and inhibits a specified kallikrein with K. of about 20 nM or less. "Inhibition" refers to blocking the catalytic activity of kallikrein and so is measurable in vitro in assays using chromogenic or fluorogenic substrates or in assays involving macromolecules.

Amino-acid residues are discussed in three ways: full origin are likely to be non-immunogenic in humans. The 45 name of the amino acid, standard three-letter code, and standard single-letter code. The text uses full names and three-letter code where clarity requires.

	A = Ala	G = Gly	M = Met	S = Ser
50	C = Cys	H = His	N = Asn	T = Thr
	$\mathbf{D} = \mathbf{A}\mathbf{s}\mathbf{p}$	I = De	P = Pro	V = Val
	$\mathbf{E} = \mathbf{Gh}$	K = Lys	Q = Gln	W = Trp
	$\mathbf{F} = \mathbf{Phe}$	L = Leu	R = Arg	Y = Tyr

For the purposed of this invention, "substantially homologous" sequences are at least 51%, more preferably at least 80%. identical. over any specified regions. For this invention, "substantially homologous" includes exact identity. Sequences would still be "substantially homologous" if plasma kallikrein is benign and thus, inhibition of plasma 60 within one region of at least 20 amino acids they are sufficiently similar (51% or more) but outside the region of comparison they differed totally. An insertion of one amino acid in one sequence relative to the other counts as one mismatch. Most preferably, no more than six residues, other than at termini, are different. Preferably, the divergence in sequence, particularly in the specified regions, is in the form of "conservative modifications".

- "Conservative modifications" are defined as
- (a) conservative substitutions of amino acids as defined in Table 9: and
- (b) single or multiple insertions or deletions of amino acids at termini, at domain boundaries, in loops, or in other segments of relatively high mobility.

Preferably, except at termini, no more than about six amino acids are inserted or deleted at any locus, and the modifications are outside regions known to contain important binding sites.

### **Kunitz Domains**

Herein. "Kunitz domain" and "KuDom" are used interchangeably to mean a homologue of BPTI (not of the Kunitz soya-bean trypsin inhibitor). A KuDom is a domain of a 15 protein having at least 51 amino acids (and up to about 61 amino acids) containing at least two, and preferably three, disulfides. Herein, the residues of all Kunitz domains are numbered by reference to BPII (i.e. residues 1-58, aminoacid sequence in Table 2). Thus the first cysteine residue is 20 residue 5 and the last cysteine is 55. An amino-acid sequence shall, for the purposes of this invention, be deemed a Kunitz domain if it can be aligned, with three or fewer mismatches. to the sequence shown in Table 14. An insertion or deletion matches any amino acid and "X" matches the types listed for that position Disulfide bonds link at least two of: 5 to 55, 14 to 38, and 30 to 51. The number of disulfides may be reduced by one, but none of the standard cysteines shall be left unpaired. Thus, if one cysteine is changed, then a compen- 30 sating cysteine is added in a suitable location or the matching cysteine is also replaced by a non-cysteine (the latter being generally preferred). For example, Drosophila funebris male accessory gland protease inhibitor has no cysteine at position 5, but has a cysteine at position -1 (just before 35 position 1); presumably this forms a disulfide to CYS55. If Cys<sub>14</sub> and Cys<sub>38</sub> are replaced, the requirement of Gly,<sub>12</sub>, (Gly or Scr)37, and Gly36 are dropped. From zero to many residues, including additional domains (including other

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Protease inhibitors, such as Kunitz domains, function by binding into the active site of the protease so that a peptide 45 bond (the "scissile bond") is: 1) not cleaved, 2) cleaved very slowly, or 3) cleaved to no effect because the structure of the inhibitor prevents release or separation of the cleaved segments. In Kunitz domains, disulfide bonds act to hold the protein together even if exposed peptide bonds are cleaved. 50 From the residue on the amino side of the scissile bond, and moving away from the bond, residues are conventionally called P1, P2, P3, etc. Residues that follow the scissile bond are called P1', P2', P3', etc. (SCHE67, SCHE68). It is generally accepted that each serine protease has sites 55 (comprising several residues) S 1. S2, etc. that receive the side groups and main-chain atoms of residues P1. P2, etc. of the substrate or inhibitor and sites S1', S2', etc. that receive the side groups and main-chain atoms of P1', P2', etc. of the substrate or inhibitor. It is the interactions between the S 60 ING sites and the P side groups and main chain atoms that give the protease specificity with respect to substrates and the inhibitors specificity with respect to proteases. Because the fragment having the new amino terminus leaves the protease first, many worker designing small molecule protease inhibi- 65 tors have concentrated on compounds that bind sites S1. S2.

LASK80 reviews protein protease inhibitors. Some inhibitors have several reactive sites on one polypeptide chain, and these domains usually have different sequences. specificities, and even topologies. It is known that substituting amino acids in the P5 to P5' region influences the specificity of an inhibitor. Previously, attention has been focused on the PI residue and those very close to it because these can change the specificity from one enzyme class to another. LASK80 suggests that among KuDoms, inhibitors with P1=Lys or Arg inhibit trypsin, those with P1=Tyr, Phe, Trp. Leu and Met inhibit chymotrypsin, and those with P1=Ala or Ser are likely to inhibit clastase. Among the Kazal inhibitors, LASK80 continues, inhibitors with P1=Leu or Met are strong inhibitors of elastase, and in the Bowman-Kirk family elastase is inhibited with P1=Ala, but not with P1=Leu. Such limited changes do not provide inhibitors of truly high affinity (i.e. better than 1 to 10 nM).

KuDoms are defined above. The 3D structure (at high resolution) of BPTI (the archetypal Kunitz domain) is known. One of the X-ray structures is deposited in the Brookhaven Protein Data Bank as "6PTI"]. The 3D structure of some BPTI homologues (EIGE90, HYNE90) are known. At least seventy KuDom sequences are known. Known human homologues include three KuDoms of LACI (WUNT88, GERA89, NOVO89), two KuDoms of Inter-aof one residue shall count as one mismatch. In Table 14, "x" 25 Trypsin Inhibitor. APP-I (KIDO88). a KuDom from collagen, and three KuDoms of TFPI-2 (SPRE94).

Lipoprotein-associated coagulation inhibitor (LACI) is a human serum phosphoglycoprotein with a molecular weight of 39 kDa (amino-acid sequence in Table 1) containing three KuDoms. We refer hereinafter to the protein as LACI and to the Kunitz domains thereof as LACI-K1 (residues 50 to 107), LACI-K2 (residues 121 to 178), and LACI-K3 (213 to 270). The cDNA sequence of LACI is reported in WUNT88. GIRA89 reports mutational studies in which the P1 residues of each of the three KuDoms were altered. LACI-K1 inhibits Factor VIIa (F.VII<sub>a</sub>) when F.VII<sub>a</sub> is complexed to tissue factor and LACI-K2 inhibits Factor X<sub>o</sub>. It is not known whether LACI-K3 inhibits anything. Neither LACI nor any KuDoms), can be attached to either end of a Kunitz domain. 40 of the KuDoms of LACI is a potent plasma kallikrein inhibitor.

In one preferred embodiment of this invention. KuDoms are substantially homologous with LACI-K1, but differ in ways that confer strong plasma kallikrein inhibitory activity discussed below. Other KuDoms of this invention are homologous to other naturally-occurring KuDoms, particularly to other human KuDoms. For use in humans, the proteins of this invention are designed to be highly similar in sequence to one or another human KuDom to reduce the risk of causing an immune response.

Variegation of a protein is typically achieved by preparing a correspondingly variegated mixture of DNA (with variable codons encoding variable residues). cloning it into suitable vectors, and expressing the DNA in suitable host cells. For any given protein molecule of the library, the choice of amino acid at each variable residue, subject to the above constraints, is random, the result of the happenstance of which DNA expressed that protein molecule.

FIRST LACI-KI LIBRARY SCREENED FOR PKA BIND-

Applicants have screened a first large library of LACI-K1 domains (patern of variegation is shown in Table 21), with the results shown in Table 3. In Table 3. "Library Residues" are those permitted to occur, randomly, at that position, in the library, and "Preferred Residues" are those appearing at that position in at least one of the 10 variants identified as binding to human kallikrein.

At residues 13, 16, 17, 18, 31, and 32, the selections are very strong. At position 34, the selection for either SER or THR is quite strong. At position 39, the selection for GLY is strong. Position 19 seems to be rather tolerant.

It should be appreciated that Applicants have not 5 sequenced all of the positive isolates in this or other the libraries herein disclosed, that some of the possible mutant proteins may not have been present in the library in detectable amounts, and that, at some positions, only some of the possible amino acids were intended to be included in the 10 library.

### SECOND LIBRARY OF LACI-K1 and SELECTION OF **NEW KALLIKREIN INHIBITORS**

Applicants prepared a second LACI-K1 library as shown in Table 750. This library utilized the observation of the first 15 selection and allows variability at positions 10, 11, 13, 15, 16, 17, 18, 19, and 21. The residues at positions 34 and 39 were fixed at S<sub>34</sub> and G<sub>39</sub>. Selectants KK2/#1 through KK2/#13, as shown in Table 2 were obtained in the same manner as described in the Example section for the first 20 screeneing. Applicants prepared the proteins KK2/#11 and KK2/#13 in S. cerevisiae in the Matot system described herein. Preliminary measurements indicate that these proteins are very potent pKA inhibitors with K less than 300 pM and probably less than 100 pM.

Using the selected sequences and the binding data of selected KuDoms, we can write a recipe for a high-affinity pKA-inhibiting KuDom that can be applied to other human KuDom parentals. First, the KuDom must meet the requirements in Table 14. The substitutions shown in Table 15 are 30 likely to confer high-affinity pKA inhibitory activity on any KuDom. Thus a protein that contains a sequence that is a KuDom, as shown in Table 14, and that contains at each of the position 12-21 and 32-39 an amino-acid type shown in human pKA. More preferably, the protein would have an amino-acid type shown in Table 15 for all of the positions listed in Table 15. To reduce the potential for immune response, one should use one or another human KuDom as parental protein to give the sequence outside the binding 40 region.

It is likely that a protein that comprises an amino-acid sequence that is substantially homologous to one of KK2/ #13, KK2/#11, or KKII/3#6 from residue 5 through residue 55 (as shown in Table 2) and is identical to one of KK2#13, 45 KK2/#11, or KKII/3#6 at positions 13-19, 31, 32, 34, and 39 will inhibit human pKA with a K, of 5 nM or less. KK2/#13, KK2/#11, and KKII/3#6 differs from LACI-K1 at 10, 8, and 7 positions respectively. It is not clear that these substitutions are equally important in fostering pKA binding and 50 inhibition. From the known pKA inhibitors listed, one can prepare a series of molecules that are progressively reverted toward LACI-K1. It is expected that the molecules will show less affinity for pKA but also less potential for antigenicity. A person skilled in the art can pick a protein of sufficient 55 potency and low immunogenicity from this collection. It is also possible that substitutions in one of the listed pKA inhibitors by amino acids that differ from LACI-K1 can reduce the immunogenicity without reducing the affinity for pKA to a degree that makes the protein unsuitable for use as 60

#### **DESIGNED KuDom PKA Inhibitors**

Hereinaster, "DKI" will mean a "Designed PKA Inhibitor" that are KuDoms that incorporate amino-acid sequence information from the SPI series of molecules. especially 65 KK2/#13. KK2/#11. or KKII/3#6. Sequences of several DKIs and their parental proteins are given in Table 2.

Hereinafter, the statement "the mutations  $X_{nn}Y_1$ ,  $X_{nn}Y_2$ , ... may not be needed" means that each of the mutations might be separately found to be unnecessary. That is, the list is not to be taken as a block to be applied together, but as a list of things to be tested. Similarly, the lists of additional mutations are to be tested singly.

Protein DKI-1.2.1 is based on human LACI-K2 and shown in Table 2. The mutations P11G, I13R, Y17A, I18H, T19P. Y21W, R32E, K34S, and L39G are likely to confer high affinity for pKA. Some of these substitutions may not be necessary; in particular, P11G and T19P may not be necessary. Other mutations that might improve the pKA affinity include E9A. D10E. G16A. Y21F. and L39E.

Protein DKI-1.3.1 (Table 2) is based on human LACI-K3. The mutations R11D, L13P, N17A, E18H, N19P, R31E, K34S, and S36G are intended to confer high affinity for pKA. Some of these substitutions may not be necessary; in particular. N19P may not be necessary. Other changes that might improve K<sub>D</sub> include D10E, F21W and G39E.

Protein DKI-2.1 (Table 2) is a based on the human collagen 03 KuDom. The mutations D16A, F17A, I18H. R32E, and W34S are likely to confer high affinity for pKA. Some of these substitutions may not be necessary; in particular, R32E may not be necessary. Other mutations that might improve the pKA affinity include K9A, D10E, D 16G, 25 K20R, R32T, W34V, and G39E.

DKI-3.1.1 (Table 2) is derived from Human TFPI-2 domain 1. The exchanges Y11G, L17A, L18H, R31E, and L34S are likely to confer high affinity for pKA. The mutation L34S may not be needed. Other mutations that might foster pKA binding include Y21W, Y21F, Q32E, L34T. L34L and E39G.

DKI-3.2.1 (Table 2) is derived from Human TFPI-2 domain 2. This parental domain contains insertions after residue 9 (one residue) and 42 (two residues). The mutations Table 15 for that position is likely to be a potent inhibitor of 35 E15R, G16A, S 17A, T18H,E19P, K32T, and F34V are intended to confer affinity for pKA. If one needs a pKA inhibitor based on TFPI domain 2, a preferred route is to make a library of domains allowing the substitutions given and many others and then select binders.

> DKI-33.1 (Table 2) is derived from human TFPI-2, domain 3. The substitutions L13H, S 15R, and N17A are likely to confer high affinity for pKA. Other mutations that might foster pKA binding include D10E, T19Q, Y21W, T36G, and G39E.

> DKI-4.1.1 (Table 2) is from human ITI-K1 by assertion of S10D, M15R, M17A, T18H, Q34S, and M39G. The mutations M39G and Q34V may not be necessary. Other mutations that should foster pKA binding include: G16A, M17N. S19Q, Y21W, and Y2IF.

DKI-4.2.1 (Table 2) is from human ITI-K2 through the mutations V10D, R11D, F17A, I18H, V31E, L32E, P34S, and Q39E. The mutations V31E, L32E, and Q39E might not be necessary. Other mutation that should foster pKA binding include: V10E, Q19P, L20R, W21F, P34L, and Q39G. DKI-4.2.2 has eight mutations: V10D, R11D, F17A, I18H, L20R. V31E, L32E, and P34S.

DKI-5.1 is derived from human APP-I (also known as Protease Nexin-II) by mutations M17A, I18H, S19P, A31E, and P32E and is likely to be a potent pKA inhibitor. The mutations S19P, A3 1E, and P32E many not be needed. Other mutations that might foster pKA binding include TIID.

DKI-6.1 is derived from the HKI B9 KuDom (NORR93) by the five substitutions: K11D. Q15R. T16A. M17A. M18H, T19P, and L32E. DKI-6.1 is likely to be a potent pKA inhibitor. The mutations L32E, and T19P might not be needed.

Although BPII is not an especially good pKA inhibitor. it could be made into one. DKI-7.1 is derived from BPTI by the mutations Y10E, K15R, R17A, R118H, I19P, Q31E, T32E, and R39E which is likely to increase the affinity for pKA. The mutations Y10E, K15R, I19P, Q31E, T32E, and R39E may not be needed; the really important mutations are R17A and R118H.

### MODIFICATION OF KUNITZ DOMAINS

KuDoms are quite small; if this should cause a pharmacological problem, such as excessively quick elimination from 10 circulation, two or more such domains may be joined. A preferred linker is a sequence of one or more amino acids. A preferred linker is one found between repeated domains of a human protein, especially the linkers found in human BPTI homologues, one of which has two domains (BALD85, ALBR83a, ALBR83b) and another of which has three (WUNT88). Peptide linkers have the advantage that the entire protein may then be expressed by recombinant DNA techniques. It is also possible to use a nonpeptidyl linker. such as one of those commonly used to form immunogenic 20 conjugates. An alternative means of increasing the serum residence of a BPTI-like KuDom is to link it to polyethyleneglycol, so called PEGylation (DAVI79).

WAYS TO IMPROVE SPECIFICITY OF, FOR EXAMPLE, KKII/3#7, KK2/#11, AND KK2/#13 FOR PLASMA KALLIKREIN:

Because we have made a large part of the surface of KKII/3#6. KK2/#11. and KK2/#13 complementary to the surface of pKA, R.15 is not essential for specific binding to pKA. Many of the enzymes in the clotting and fibrinolytic 30 DNA technology include Watson et al., Molecular Biology pathways cut preferentially after Arg or Lys. Not having a basic residue at the P1 position may give rise to greater specificity. The variant KKII/3#7-K15A (shown in Table 27). having an ALA at P1, is likely to be a good pKA inhibitor and may have higher specificity for pKA relative to 35 other proteases than doesKKII/3#7. The affinity of KKII/ 3#7-K15A for pKA is likely to be less than the affinity of KKII/3#7 for pKA, but the loss of affinity for other Arg/ Lys-preferring enzymes is likely to be greater and, in many Other mutants that are likely to have good affinity and very high specificity include KK2/#13-R15A and KK2/#11-R15S. This approach could be applied to other high-affinity pKA inhibitors.

### MODE OF PRODUCTION

The proteins of this invention may be produced by any conventional technique, including

- (a) nonbiological synthesis by sequential coupling of component amino acids,
- (b) production by recombinant DNA techniques in a 50 suitable host cell, and
- (c) removal of undesired sequences from LACI and coupling of synthetic replacement sequences

The proteins disclosed herein are preferably produced, recombinantly, in a suitable host, such as bacteria from the 55 sized BPTI and a homologue eighteen years ago. genera Bacillus, Escherichia, Salmonella, Erwinia, and yeasts from the genera Hansenula, Kluyveromyces, Pichia, Rhinosporidium, Saccharomyces, Schizosaccharomyces, or cultured mammalian cells such as COS-1. The more preferred hosts are microorganisms of the 60 species Pichia pastoris, Bacillus subtilis, Bacillus brevis, Saccharomyces cerevisiae, Escherichia coli and Yarrowia lipolytica. Any promoter, regulatable or constitutive, which is functional in the host may be used to control gene expression.

Preferably the proteins are secreted. Most preferably, the proteins are obtained from conditioned medium. It is not required that the proteins described herein be secreted. Secretion is the preferred route because proteins are more likely to fold correctly, can be produced in conditioned medium with few contaminants, and are less likely to be toxic to host cells. Secretion is not required.

Unless there is a specific reason to include glycogroups, we prefer proteins designed to lack N-linked glycosylation sites to reduce potential for antigenicity of glycogroups and so that equivalent proteins can be expressed in a wide variety of organisms including: 1) E. coli, 2) B. subtilis, 3) P. pastoris. 4) S. cerevisiae, and 5) mammalian cells.

Several means exist for reducing the problem of host cells producing proteases that degrade the recombinant product; see, inter alia BANE90 and BANE91. VAND92 reports that overexpression of the B. subtilis signal peptidase in E. coli, leads to increased expression of a heterologous fusion protein. ANBA88 reports that addition of PMSF (a serine proteases inhibitor) to the culture medium improved the yield of a fusion protein.

Other factors that may affect production of these and other proteins disclosed herein include: 1) codon usage (optimizing codons for the host is preferred). 2) signal sequence, 3) amino-acid sequence at intended processing sites, presence and localization of processing enzymes. 25 deletion, mutation, or inhibition of various enzymes that might alter or degrade the engineered product and mutations that make the host more permissive in secretion (permissive secretion hosts are preferred).

Reference works on the general principles of recombinant of the Gene, Volumes I and II, The Benjamin/Cummings Publishing Company, Inc., Menlo Park, Calif. (1987); Darnell et al., Molecular Cell Biology, Scientific American Books, Inc., New York, N.Y. (1986); Lewin, Genes II, John Wiley & Sons, New York, N.Y. (1985); Old, et al., Principles of Gene Manipulation: An Introduction to Genetic Engineering, 2d edition, University of California Press, Berkeley, Calif. (1981); Sambrook et al, Molecular Cloning: applications, specificity is more important than affinity. 40 Spring Harbor, N.Y. (1989); and Ausubel et al, Current Protocols in Molecular Biology. Wiley Interscience, N.Y., (1987, 1992). These references are herein entirely incorporated by reference as are the references cited therein. PREPARATION OF PEPTIDES

> Chemical polypeptide synthesis is a rapidly evolving area in the art, and methods of solid phase polypeptide synthesis are well-described in the following references, hereby entirely incorporated by reference: (Merrifield, J Amer Chem Soc 85:2149-2154 (1963); Merrifield, Science 232:341-347 (1986); Wade et al., Biopolymers 25:S21-S37 (1986); Fields, Int J Polypeptide Prot Res 35:161 (1990); MilliGen Report Nos. 2 and 2a, Millipore Corporation, Bedford, Mass, 1987) Ausubel et al, supra, and Sambrook et al, supra. Tan and Kaiser (Biochemistry, 1977, 16:1531-41) synthe-

As is known in the art, such methods involve blocking or protecting reactive functional groups, such as free amino, carboxyl and thio groups. After polypeptide bond formation, the protective groups are removed. Thus, the addition of each amino acid residue requires several reaction steps for protecting and deprotecting. Current methods utilize solid phase synthesis, wherein the C-terminal amino acid is covalently linked to an insoluble resin particles that can be filtered. Reactants are removed by washing the resin par-65 ticles with appropriate solvents using an automated machine. Various methods, including the "tBoc" method and the "Fmoc" method are well known in the art. See. inter alia.

Atherton et al., J Chem Soc Perkin Trans 1:538-546 (1981) and Sheppard et al. Int J Polypeptide Prot Res 20:451-454 (1982).

### ASSAYS FOR PLASMA KALLIKREIN BINDING AND INHIBITION

Any suitable method may be used to test the compounds of this invention. Scatchard (Ann NY Acad Sci (1949) 51:660-669) described a classical method of measuring and analyzing binding which is applicable to protein binding. This method requires relatively pure protein and the ability 10 the art. to distinguish bound protein from unbound.

A second appropriate method of measuring  $K_D$  is to measure the inhibitor activity against the enzyme. If the KD to be measured is in the 1 nM to 1 µM range, this method requires chromogenic or fluorogenic substrates and tens of 15 micrograms to milligrams of relatively pure inhibitor. For the proteins of this invention, having  $K_D$  in the range 5 nM to 50 pM, nanograms to micrograms of inhibitor suffice. When using this method, the competition between the inhibitor and the enzyme substrate can give a measured  $K_i$  20 that is higher than the true Ki. Measurement reported here are not so corrected because the correction would be very small and the any correction would reduce the K. Here, we use the measured  $K_i$  as a direct measure of  $K_D$ .

A third method of determining the affinity of a protein for 25 Goodman, supra, Avery, supra and Ebadi, supra. a second material is to have the protein displayed on a genetic package, such as M13, and measure the ability of the protein to adhere to the immobilized "second material". This method is highly sensitive because the genetic packages can be amplified. We obtain at least semiquantitative values for 30 the binding constants by use of a pH step gradient. Inhibitors of known affinity for the protease are used to establish standard profiles against which other phage-displayed inhibitors are judged. Any other suitable method of measuring protein binding may be used.

Preferably, the proteins of this invention have a Kp for pKA of at most about 5nM, more preferably at most about 300 pM, and most preferably 100 pM or less. Preferably, the binding is inhibitory so that  $K_i$  is the same as  $K_D$ . The  $K_i$  of KKII/3#6 is about 300 pM and the K<sub>i</sub>s of KK2/#11 and 40 KK2/#13 are less than 300 pM and probably less than 100

### Pharmaceutical Methods and Preparations

The preferred subject of this invention is a mammal. The invention is particularly useful in the treatment of humans. 45 but is suitable for veternary applications too.

Herein. "protection" includes "prevention". "suppression", and "treatment". "Prevention" involves administration of drug prior to the induction of disease. "Suppression" involves administration of drug prior to the 50 clinical appearance of disease. "Treatment" involves administration of drug after the appearance of disease.

In human and veterinary medicine, it may not be possible to distinguish between "preventing" and "suppressing" since the inductive event(s) may be unknown or latent, or the 55 patient is not ascertained until after the occurrence of the inductive event(s). We use the term "prophylaxis" as distinct from "treatment" to encompass "preventing" and "suppressing". Herein, "protection" includes "prophylaxis". Protection need not be absolute to be useful.

Proteins of this invention may be administered, by any means, systemically or topically, to protect a subject against a disease or adverse condition. For example, administration of such a composition may be by any parenteral route, by bolus injection or by gradual perfusion. Alternatively, or 65 concurrently, administration may be by the oral route. A suitable regimen comprises administration of an effective

amount of the protein, administered as a single dose or as several doses over a period of hours, days, months, or years.

The suitable dosage of a protein of this invention may depend on the age, sex, health, and weight of the recipient, 5 kind of concurrent treatment, if any, frequency of treatment, and the desired effect. However, the most preferred dosage can be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation by adjustment of the dose in ways known in

For methods of pre clinical and clinical testing of drugs. including proteins, see, e.g., Berkow et al, eds., The Merck Manual, 15th edition, Merck and Co., Rahway, N.J., 1987; Goodman et al. eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, Md. (1987), Ebadi, Pharmacology, Little, Brown and Co., Boston, (1985), which references and references cited there are hereby incorporated by reference.

In addition to a protein here disclosed, a pharmaceutical composition may contain pharmaceutically acceptable carriers, excipients, or auxiliaries. See, e.g., Berker, supra,

In Vitro Diagnostic Methods and Reagents

Proteins of this invention may be applied in vitro to any suitable sample that might contain plasma kallikrein to measure the pKA present. To do so, the assay must include a Signal Producing System (SPS) providing a detectable signal that depends on the amount of pKA present. The signal may be detected visually or instrumentally. Possible signals include production of colored, fluorescent, or luminescent products, alteration of the characteristics of absorption or emission of radiation by an assay component or product, and precipitation or agglutination of a component or product.

The component of the SPS most intimately associated with the diagnostic reagent is called the "label". A label may be, e.g., a radioisotope, a fluorophore, an enzyme, a co-enzyme, an enzyme substrate, an electron-dense compound, or an agglutinable particle. A radioactive isotope can be detected by use of, for example, a  $\gamma$  counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful are 3H. 125L, 131L, 35S, 14C, and preferably, <sup>125</sup>L It is also possible to label a compound with a fluorescent compound. When the fluorescently labeled compound is exposed to light of the proper wave length, its presence can be detected. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycocrythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine. Alternatively, fluorescence-emitting metals, such as 125Eu or other lanthanide, may be attached to the binding protein using such metal chelating groups as diethylenetriaminetetraacetic acid or ethylenediamine-tetraacetic acid. The proteins also can be detectably labeled by coupling to a cheminuminescent compound, such as luminol, isolumino, theromatic acridinium ester, imidazole, acridinium salt, and 60 oxalate ester. Likewise, a bioluminescent compound, such as luciferin, luciferase and aequorin, may be used to label the binding protein. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Enzyme labels, such as horseradish peroxidase and alkaline phosphatase, are preferred.

There are two basic types of assays: heterogeneous and homogeneous. In heterogeneous assays, binding of the affinity molecule to analyte does not affect the label; thus, to determine the amount of analyte, bound label must be separated from free label. In homogeneous assays, the interaction does affect the activity of the label, and analyte can be measured without separation.

In general, a kallikrein-binding protein (KBP) may be used diagnostically in the same way that an anti-pKA antibody is used. Thus, depending on the assay format, it may be used to assay pKA, or, by competitive inhibition, other substances which bind pKA.

The sample will normally be a biological fluid, such as blood, urine, lymph, semen, milk, or cerebrospinal fluid, or a derivative thereof, or a biological tissue, e.g., a tissue section or homogenate. The sample could be anything. If the sample is a biological fluid or tissue, it may be taken from a human or other mammal, vertebrate or animal, or from a plant. The preferred sample is blood, or a fraction or derivative thereof

In one embodiment, the pKA-binding protein (KBP) is immobilized, and pKA in the sample is allowed to compete with a known quantity of a labeled or specifically labelable pKA analogue. The "pKA analogue" is a molecule capable of competing with pKA for binding to the KBP, which includes pKA itself It may be labeled already, or it may be labeled subsequently by specifically binding the label to a moiety differentiating the pKA analogue from pKA. The phases are separated, and the labeled pKA analogue in one phase is quantified.

In a "sandwich assay", both an insolubilized pKA-binding agent (KBA), and a labeled KBA are employed. The pKA analyte is captured by the insolubilized KBA and is tagged by the labeled KBA, forming a tertiary complex. The reagents may be added to the sample in any order. The KBAs may be the same or different, and only one KBA need be a KBP according to this invention (the other may be, e.g., an antibody). The amount of labeled KBA in the tertiary complex is directly proportional to the amount of pKA in the sample.

The two embodiments described above are both heterogeneous assays. A homogeneous assay requires only that the label be affected by the binding of the KBP to pKA. The pKA analyte may act as its own label if a pKA inhibitor is used as a diagnostic reagent.

A label may be conjugated, directly or indirectly (e.g., through a labeled anti-KBP antibody), covalently (e.g., with SPDP) or noncovalently, to the pKA-binding protein, to produce a diagnostic reagent. Similarly, the pKA binding protein may be conjugated to a solid phase support to form a solid phase ("capture") diagnostic reagent. Suitable supports include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, and magnetite. The carrier can be soluble to some extent or insoluble for the purposes of this invention. The support material may have any structure so long as the coupled molecule is capable of binding pKA.

### In Vivo Diagnostic Uses

A Kunitz domain that binds very tightly to pKA can be used for in vivo imaging. Diagnostic imaging of disease foci was considered one of the largest commercial opportunities for monoclonal antibodies, but this opportunity has not been achieved. Despite considerable effort, only two monoclonal antibody-based imaging agents have been approved. The disappointing results obtained with monoclonal antibodies is due in large measure to:

- i) Inadequate affinity and/or specificity;
- ii) Poor penetration to target sites;
- iii) Slow clearance from nontarget sites;

iv) Immunogenicity (most are murine); and

v) High production cost and poor stability.

These limitations have led most in the diagnostic imaging field to begin to develop peptide-based imaging agents. While potentially solving the problems of poor penetration and slow clearance, peptide-based imaging agents are unlikely to possess adequate affinity, specificity and in vivo stability to be useful in most applications.

Engineered proteins are uniquely suited to the requirements for an imaging agent. In particular the extraordinary affinity and specificity that is obtainable by engineering small, stable, human-origin protein domains having known in vivo clearance rates and mechanisms combine to provide earlier, more reliable results, less toxicity/side effects, lower production and storage cost, and greater convenience of label preparation. Indeed, it should be possible to achieve the goal of realtime imaging with engineered protein imaging agents. Thus, a Kallikrein-binding protein, e.g., KKII/3#6, KK2/#11, and KK2/#13 may be used for localizing sites of excessive pKA activity.

Radio-labelled binding protein may be administered to the human or animal subject. Administration is typically by injection. e.g.. intravenous or arterial or other means of administration in a quantity sufficient to permit subsequent dynamic and/or static imaging using suitable radio-detecting devices. The dosage is the smallest amount capable of providing a diagnostically effective image, and may be determined by means conventional in the art, using known radio-imaging agents as guides.

Typically, the imaging is carried out on the whole body of the subject, or on that portion of the body or organ relevant to the condition or disease under study. The radio-labelled binding protein has accumulated. The amount of radio-labelled binding protein accumulated at a given point in time in relevant target organs can then be quantified.

A particularly suitable radio-detecting device is a scintillation camera, such as a  $\gamma$  camera. The detection device in the camera senses and records (and optional digitizes) the radioactive decay. Digitized information can be analyzed in any suitable way, many of which are known in the art. For example, a time-activity analysis can illustrate uptake through clearance of the radio-labelled binding protein by the target organs with time.

Various factors are taken into consideration in picking an appropriate radioisotope. The isotope is picked: to allow good quality resolution upon imaging, to be safe for diagnostic use in humans and animals, and, preferably, to have a short half-life so as to decrease the amount of radiation received by the body. The radioisotope used should preferably be pharmacologically inert, and the quantities administered should not have substantial physiological effect. The binding protein may be radio-labelled with different isotopes of iodine, for example <sup>123</sup>I, <sup>125</sup>I, or <sup>131</sup>I (see, for example, U.S. Pat. No. 4.609.725). The amount of labeling must be suitably monitored.

In applications to human subjects, it may be desirable to use radioisotopes other than 125 I for labelling to decrease the total dosimetry exposure of the body and to optimize the detectability of the labelled molecule. Considering ready clinical availability for use in humans. preferred radio-labels include: 99m Ic. 67 Ga. 68 Ga. 90 Y. 111 In. 113m In. 123 I. 186 Re. 60 188 Re or 211 At. Radio-labelled protein may be prepared by various methods. These include radio-halogenation by the chloramine-T or lactoperoxidase method and subsequent purification by high pressure liquid chromatography, for example, see Gutkowska et al in "Endocrinology and 65 Metabolism Clinics of America: (1987) 16 (1): 183. Other methods of radio-labelling can be used, such as IODO-BEADS™.

A radio-labelled protein may be administered by any means that enables the active agent to reach the agent's site of action in a mammal. Because proteins are subject to digestion when administered orally, parenteral administration, i.e., intravenous subcutaneous, 5 intramuscular, would ordinarily be used to optimize absorption.

High-affinity, high-specificity inhibitors are also useful for in vitro diagnostics of excess human pKA activity. Other Uses

The kallikrein-binding proteins of this invention may also be used to purify kallikrein from a fluid, e.g., blood. For this, the KBP is preferably immobilized on a support. Such supports, include those already mentioned as useful in preparing solid phase diagnostic reagents.

Proteins can be used as molecular weight markers for reference in the separation or purification of proteins. Proteins may need to be denatured to serve as molecular weight markers. A second general utility for proteins is the use of hydrolyzed protein as a nutrient source. Proteins may also be used to increase the viscosity of a solution.

The proteins of this invention may be used for any of the foregoing purposes, as well as for therapeutic and diagnostic purposes as discussed further earlier in this specification.

#### **EXAMPLE 1:**

### Construction of First LACI-K1 Library

A synthetic oligonucleotide duplex having NsiI- and MluI-compatible ends was cloned into a parental vector (LACI:III) previously cleaved with the above two enzymes. The resultant ligated material was transfected by electropo- 30 ration into XLIMR (F-) Escherichia coli strain and plated on Amp plates to obtain phage-generating Ap<sup>R</sup> colonies. The variegation scheme for Phase 1 focuses on the P1 region. and affected residues 13, 16, 17, 18 and 19. It allowed for 6.6×10° different DNA sequences (3.1×10° different protein sequences). The library obtained consisted of 1.4×10<sup>5</sup> independent cfu's which is approximately a two fold representation of the whole library. The phage stock generated from this plating gave a total titer of 1.4×10<sup>13</sup> pfu's in about 3.9 ml. with each independent clone being represented, on average. 1×10<sup>7</sup> in total and 2.6×10<sup>6</sup> times per ml of phage <sup>40</sup> stock.

To allow for variegation of residues 31, 32, 34 and 39 (phase II), synthetic oligonucleotide 5 duplexes with MluI-and BstEII- compatible ends were cloned into previously cleaved R, DNA derived from one of the following

- i) the parental construction.
- ii) the phase I library, or
- iii) display phage selected from the first phase binding to a given target.

The variegation scheme for phase II allows for 4096 different DNA sequences (1600 different protein sequences) due to alterations at residues 31, 32, 34 and 39. The final phase II variegation is dependent upon the level of variegation remaining following the three rounds of binding and elution with a given target in phase I.

The combined possible variegation for both phases equals  $2.7 \times 10^8$  different DNA sequences or  $5.0 \times 10^7$  different protein sequences. When previously selected display phage are used as the origin of  $R_7$  DNA for the phase II variegation, the final level of variegation is probably in the range of  $10^5$  to  $60^{10}$ .

### **EXAMPLE 2:**

### Screening of LACI (K1) Library for Binding to Kallikrein

The overall scheme for selecting a LACI-K1 variant to bind to a given protease involves incubation of the phagedisplay library with the kallikrein-beads of interest in a buffered solution (PBS containing 1 mg/ml BSA) followed by washing away the unbound and poorly retained display-phage variant with PBS containing 0.1% Tween 20. Kallikrein beads were made by coupling human plasma Kallikrein (Calbiochem. San Diego, Calif., #420302) to agarose beads using Reactigel (6x) (Pierce, Rockford, II. #202606). The more strongly bound display-phage are eluted with a low pH elution buffer, typically citrate buffer (pH 2.0) containing 1 mg/ml BSA, which is immediately neutralized with Tris buffer to pH 7.5. This process constitutes a single round of selection.

The neutralized eluted display-phage can be either used:
i) to inoculate an F<sup>+</sup> strain of E. coli to generate a new
display-phage stock, to be used for subsequent rounds
of selection (so-called conventional screening), or

ii) be used directly for another immediate round of selection with the protease beads (so-called quick screening).

Typically, three rounds of either method, or a combination of the two, are performed to give rise to the final selected display-phage from which a representative number are sequenced and analyzed for binding properties either as pools of display-phage or as individual clones.

Two phases of selection were performed, each consisting of three rounds of binding and elution. Phase I selection used the phase I library (variegated residues 13, 16, 17, 18, and 19) which went through three rounds of binding and elution against a target protease giving rise to a subpopulation of clones. The R<sub>2</sub> DNA derived from this selected subpopulation was used to generate the Phase II library (addition of variegated residues 31, 32, 34 and 39). The 1.8×10<sup>7</sup> independent transformants were obtained for each of the phase II libraries. The phase II libraries underwent three further rounds of binding and elution with the same target protease giving rise to the final selectants.

Following two phases of selection against human plasma kallikrein-agarose beads a number (10) of the final selection display-phage were sequenced. The amino-acid sequences are shown in Table 2, entries KBPconl through KKII/3190 C.

Table 23 shows that KkII/3(D) is a highly specific inhibitor of human Kallikrein. Phage that display the LACI-K1 derivative KkII/3(D) bind to Kallikrein beads at least 50-times more than it binds to other protease targets.

Preliminary measurements indicate that KKII/3#6 is a potent inhibitor of pKA with  $K_i$  probably less than 500 pM. Expression, Purification and Kinetic Analysis.

The three isolates KKII/3#6. KK2#11, and KK2/#13 were recloned into a yeast expression vector. The yeast expression vector is derived from pMFalpha8 (KUR182 and MIYA85). The LACI variant genes were fused to part of the mato: 1 gene, generating a hybrid gene consisting of the mato: 1 promoter-signal peptide and leader sequence-fused to the LACI variant. The cloning site is shown in Table 24. Note that the correctly processed LACI-K1 variant protein should be as detailed in Table 2 with the addition of residues glu-ala-ala-glu (SEQ ID NO: 70) to the N-terminal met (residue 1 in Table 2). Expression in S. cerevisiae gave acceptable yield typical of this system. Yeast-expressed LACI (kunitz domain 1), BPTI and LACI variants: KKII/3#6. KK2/#11, and KK2/#13 were purified by affinity chromatography using trypsin-agarose beads.

For larger-scale production, *Pichia pastoris* is a preferred host. The most preferred production system in *P. pastoris* is the alcohol oxidase system. Others have produced a number of proteins in the yeast *Pichia pastoris*. For example, Vedvick et al. (VEDV91) and Wagner et al. (WAGN92) produced aprotinin from the alcohol oxidase promoter with

induction by methanol as a secreted protein in the culture medium at ~1 mg/ml. Gregg et al. (GREG93) have reviewed production of a number of proteins in *P. pastoris*. Table 1 of GREG93 shows proteins that have been produced in *P.* pastoris and the yields.

All references, including those to U.S. and foreign patents or patent applications, and to nonpatent disclosures, are hereby incorporated by reference in their entirety.

TABLE 1

Sequence of whole LACI:						
	1	5	5	5	5	5
1 51 101 151 201 251 301	HSF KKI erfik qstk	TMKKVHA CAFKADD MCTRDnan YEECIg vpsife ennitsk n (SEQ I	LWASVCLLLN GPCKAIMKRF riiktlqqee nmmfetlee fhgpsweltp qedrackkg D NO. 18)	LAPAPLNAds FFNIFTRQCE kpdfcfleed chricedgpn adrglcrane fiqriskggl	eedeehtiit EFTYGGCEGN PRICERVITE gfqvdnygtq nuflyynsvig iktkrkrkkq	chelpplkiM QNRFESLEEC yfynngkod havmslip kerpfkysge rykiayeeif

The signal sequence (1-28) is uppercase and underscored

LACI-K1 is uppercase
LACI-K2 is underscored

25

LACI-K3 is bold

TABLE 2 is below.

TABLE 3
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	Summary of first	selection of L.	ACI-KI domains f	
_	BPTI#	(Lac I)	Library Residues	Preferred Residues
0	13	P	LHPR	HP
	16	A	AG	AG
	17	1	FYLHINA SCPRTVD G	NSA
	18	M	all	HL.
	19	K	LWQMKAG SPRTVE	QLP
	31	Б	EQ	E
	32	E	EQ	EQ
	34	I	ail	STI
	39	E	ali	GEA

TABLE 2

### Sequences of Kunitz domains, some of which inhibit human pKA.

# Amino-acid sequence

klent	1134567890123456789012345678901234567890123456789012345678	SEQ ID NO.
		SEQ ID NO.
Drii LACIVI	RPDECLEPPYTGPCKARIIRYEYNAKAGLCQTFVYGGCRAKRNNEKSAEDCMRTCGGA	SEQ ID NO. 2
THE PARTY	MILE IL SILLE CONTROLLE CO	SEQ ID NO. 3
ENTERN .	missicaficaddeHckaNHQrfffinifrqcFEfSyggcGgnqmfesleeckkmctrd	SEQ ID NO. 4
THEMEN	mhsfcafkaddgHckASLPrfffniftrqcHEfflyggcEgnqurfesleeckkmctrd	SEQ ID NO. 5
WWINDHS	mhsfcafkaddgPckANHLrfffniftrqcERfSyggeGgnqnrfesleeckkmctrd	SEQ ID NO. 6
CHCMNN	mhsfcaftaddgHckANHQrfffniftrqcEEfTyggcGgnqnrfcsleeckkmctrd	SEQ ID NO. 7
WIND SHA	mhsfcafkaddgHckANHQrfffniftrqcEQfTyggcAgnqnrfesleeckkmctrd	SEQ ID NO. 8
CAC ATTORN	BIBSIC2IK20GFICKASLPHIIDHMCKFflyggrCommyfeclesablametal	SEQ ID NO. 9
CHICALAIA OHICATAIA	mhsfcafkaddgHckANHQrfffniftrqcEEfSyggcGgnqnrfcsleeckkmctrd	SEQ ID NO. 10
KKII/3#9	mhsfcaffaddgHckANHQrfffnifnqcERfSyggoGgnqnrfesleeckkmctrd	SEQ ID NO. 11
KK11/3#0	mbsfcafkaddgHckANHQrfffniftrqcEEfSyggcGgnqnrfcsleeckkmctrd	SEQ ID NO. 12
KKIIII	mhsfrafhaddgHckANHQrfffnifrqcEEfSyggeCGgnqnrfesleeckkmctrd	SEQ ID NO. 13
KKI/3(a)	mhsfeafkaddgHckGAHLrfffniftrqcEEffyggeEgnqurfesleeckkmetrd	SEQ ID NO. 14
KKI/3/h)	mhsfeafkaddgRckGAHLrfffniftrqceefiyggcegnqnrfesleeckkmctrd	SEQ ID NO. 15
KKU3(U)	mhsfeafkaddgPckAIHI_rfffniftrqceefiyggcegncpurfesleeckkmctrd	SEQ ID NO. 16
EEDOW.	mhsfcaffraddgHckANHQrfffnifrqcHEfSyggcGgnqnrfesleeckkmctrd	SEQ ID NO. 17
KKU#15	mbsfcafkaDGgRcRGAHPrWffniftrqcEEfSyggcGgnqmfcsleeckkmctrd	SEQ ID NO. 19
VV7414	IBISICALEAD GERCKGAHPT Wiftinftrock FEStygge Gongrefee least travel	SEQ ID NO. 20
VP/N42	mnsicalkaDDaPckAAHPrWffniftrockEfSvoocGovernefesleeshtenend	SEQ ID NO. 21
KK2#II 1	misicalkaDDaPcRAAHPrWffniftmcFFfSvagecConcentral and the send	SEQ ID NO. 22
KK2/#1 :	mbsfcaftaDVgRcRGAHPrWffniftrqcHEfSyggcGgnqnrfcsleeckkmctrd	
	- See See See See See See See See See Se	SEQ ID NO. 23

### TABLE 2-continued

	Sequences of Kunitz domains, some of which inhibit human pKA.	
	Amino-acid sequence	
	111111111222222222333333333444444444555555555	
klen	1 1234567890123456789012345678901234567890123456789012345678	SEQ ID NO.
KK2/#4	mhsfcaftaDVgRcRGAQPrFffniftrqcFEfSyggeGgnqnrfesleeckkmetrd	SEQ ID NO. 2
KK2/#6	mbsfcafkaDDgScRAAHLrWffniftrocEEfSvggcGgnomfesleeckkmetri	SEQ ID NO. 2
KJK2/#10	hlsfcafkaEGgScRAAHQrWffniftrqcEEfSyggcGgpqnrfesleeckkmcml	SEQ ID NO. 2
KK2/#8	mbsfcafkaDDgPcRGAHLrFffniftrqcFEfSyggcGgnqnrfesleeckkmctrd	SEQ ID NO. 2
KK2/#3	mbsfcafkaDDgHcRGALPrWffniftrqcEEfSyggcGgnqnrfesleeckkmctrd	SEQ ID NO. 2
IKIK2/#9	mbsfcafkaDSgNcRGNLPrFffmiftrocEEfSvggcGpnomfesleecklyncml	SEO ID NO. 2
IKIK <i>2/#</i> 7	mbsfcafkaDSgRcRGNHOrFffniftrucEEfSvgocGgrunnfesleeckkmetrd	SEQ ID NO. 3
KK2/#12	mhsfcafkaDGgRcRAIOPrWffniftrocFEfSvgcGgroonrfesleeckkmend	SEQ ID NO. 3
KK2con1	mbskrafkaDDgRcRGAHPrWffniftrocEEfSyggcGgngurfesleecktrycted	SEQ ID NO. 3
Human LACI-K2	KPDFCFLEEDPGKCRGYITRYFYNNOTKOCERFKYGGCLGNMNNFFTI FFCKNICEDG	SEQ ID NO. 3
DKI-1.2.1	kpdfcflocdGeRcreAHPrWfvnnotknceRfSveerGommonfetleechnicede	SEO ID NO. 3
Human LACI-K3	GPSWCLTPADRGLCRANENRFYYNSVIGKCRPFKYSGCGGNENNFTSKOFCLRACKKG	SEQ ID NO. 3
DKI-1.3.1	gpswcltpadDgPcraAHPrfyvpsvigkcEpfSvsgcgonennftskoechacke	SEQ ID NO. 3
Human collagen 023	ETDICKLPKDBGRCRDFILKWYYDPNTKSCARFWYGGGGGNENKFGSOKECEKVCAPV	SEQ ID NO. 3
KuDom		
DKI-2.1	etdicklpkdegtcrAAHlkwyydpntkscaEfSyggcggnenkfgsqkecekvcapv	SEQ ID NO. 3
IFPI-2 DOMAIN 1	NAEKCLLPLDYGPCRALLLRYYYDRYTQSCRQFLYGGCEGNANNFYTWEACDDACWRI	SEO ID NO. 3
DKI-3.1.1	naciclipldGgpcraAHlryyydrytqscEqfSyggcegnannfytwcaoddacwri	SEQ ID NO. 4
třpi-2 DOMAIN 2	· • • • • • • • • • • • • • • • • • • •	SEQ ID NO. 4
	VDDQCEGSTAKYFFNLSSMTCEKFFSGGCHRNR-	
DKI-3.2.1	VPAVCTIQUS-	CEO TO MO
	vddqcRAAHPkyfinlssmtceEffsggchrnr-	SEQ ID NO. 4
TEDLO DOMANA 2	ienrfpdeatcmgfcapk IPSFCYSPKDEGLCSANVTRYYFNPRYRTCDAFTYTGCGGNDNNFVSREDCKRACAKA	
DKT-331	ipsfcyspkdegHcRaAHQryyfnpryncdaftytgcggndnnfvsredckracaka	SEQ ID NO. 43
HIMAN TILK	KEDSCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKECLQTCRTV	SEQ ID NO. 44
DETAIL	kedscqlgyDagpcRgAHPryfyngtsmacetfSyggcGgngmfvtekeclqtentv	SEQ ID NO. 45
Human III.K2	TVAACNLPIVRGPCRAFIQLWAFDAVKGKCVLPPYGGCQGNGNKFYSEKECREYCGVP	SEQ ID NO. 40
DRTA21	tvaacnbiDDgpcraAHqlwafdavkgkcEEfSyggcEgngnkfysckecreycgvp	SEQ ID NO. 47
DKT-4.2.2	tvascnlpiDDgpcraAHqRwafdavkgkcEEfSyggcogngnkfysekecreycgvp	SEQ ID NO. 48
HIMAN	VREVCSEQAETGPCRAMISRWYFDVTEGKCAPFFYGGGGGRRNNFDTEEYCMAVGGSA	SEQ ID NO. 49
PROTEASE	VALUE CODQUESTIC CRAIMEDRA IPD VIEUR CAPPPIGOCOGNINN PDIEBYCMAYOGSA	SEQ ID NO. 50
NEXIN-II		
	vrevcsoquetgpcraAHPrwyfdvtegkcFEfSyggeggnranfdteeyemavegsa	
HKT BQ domoin	LPNVCAFPMEKGPCQTYMTRWFFNFETGECFLFAYGGCGGNSNNFLRKEKCEKFCKFT	SEQ ID NO. 51
DKIA!	procapmeDgpcRAAHPrwfinfetgeceEfayggcggnsnnftrkekcekfckft	SEQ ID NO. 53
DK7-7 1	rpdfcleppEtgpcRaAHPryfynakaglcEEfvyggcGakmnfksaedemrtegga	SEQ ID NO. 54
DE2-7.1	rpuncteppresgpckaArteryrynakagicktetwyggeGakrimiksaedemriegga	SEQ ID NO. 55

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### **TABLE 9-continued**

Bind	ling Data for	Selected Kallikrein-bi	nding Display-Phage.		Conservative and Semiconservative substitutions						
Display	y-Phage(a)	Fraction Bound(b)	Relative Binding(c)	45	Initial	•	Conservative	Semi-conservative			
LACI		4.2 × 10 <sup>-6</sup>	1.0	•	AA type	Category	substitution	substitution.			
BPTT		$2.5 \times 10^{-5}$	6.0			slightly					
KKI/3(a	(a)	$3.2 \times 10^{-3}$	761			polar					
KKJ/3(1	b)	$2.2 \times 10^{-3}$	524	50	C	free SH	A, M, L, V, I	F, G			
KKII/3	#5	$3.9 \times 10^{-3}$	928	30		disulfide	nothing	nothing			
KKIV3	#6	8.7 × 10 <sup>-3</sup>	2071		D	acidic, hydrophilic	E, N, S, T, Q	K, R, H, A			
Clonal is	olates of displ	av-phage LACLK) is	the parental molecule, BPTI		E	acidic,	D, Q, S, T, N	K, R, H, A			
			ue parentai molecule, Br II		*	hydrophilic					
wine pano	creatic trypsin	inhibitor) is a contro	of and KKII/3 (5 and 6) and		F	hydrophilic aromatic	WYHLM	LVC			
rvine pano IV3 (a and The numl	creatic trypsir d b) were sek ber of pfu's el	inhibitor) is a contro octed by binding to the luted after a binding en	one parental monecule, BF 11 of and KKII/3 (5 and 6) and ac target protease, kallikrein. openment as a fraction of the		F G	aromatic Gly-only	W, Y, H, L, M nothing	I, V, (C) nothing			
wine pand (1/3 (a and The number out number	creatic trypsin d b) were seld ber of pfu's el er (10 <sup>10</sup> pfu's)	n inhibitor) is a contro ected by binding to the luted after a binding ex	of and KKII/3 (5 and 6) and target protease, kallikrein.			aromatic Gly-only conformation "normal"		nothing D, E, H, I, K, L, M,			
wine pand (1/3 (a and The number out number	creatic trypsin d b) were seld ber of pfu's el er (10 <sup>10</sup> pfu's)	a inhibitor) is a confre- ceted by binding to the lated after a binding en- terior of the parental displayed to the parental displayed	of and KKII/3 (5 and 6) and the target protease, kallikrein. Operiment as a fraction of the			aromatic Gly-only conformation	nothing	nothing			
wine pand (1/3 (a and The number out number	creatic trypsin d b) were seld ber of pfu's el er (10 <sup>10</sup> pfu's)	n inhibitor) is a contro ected by binding to the luted after a binding ex	of and KKII/3 (5 and 6) and the target protease, kallikrein. Operiment as a fraction of the		Ğ	aromatic Gly-only conformation "normal" conformation amphoteric aromatic aliphatic,	nothing A, S, N, T	nothing D, E, H, I, K, I., M, Q, R, V			
ovine pane GV3 (a and The number sut number Fraction	creatic trypsind b) were selecter of pfu's electer of pfu's electer (10 <sup>10</sup> pfu's) bound relative	a inhibitor) is a confre- ceted by binding to the lated after a binding en- terior of the parental displayed to the parental displayed	of and KKII/3 (5 and 6) and to target protease, kallikrein. openment as a fraction of the tay-phage, LACI-K1.	55	G H	aromatic Gly-only conformation "normal" conformation amphoteric aromatic	nothing A, S, N, T Y, F, K, R	nothing D, E, H, I, K, I, M, Q, R, V L, M, A, (C)			
ovine pane GV3 (a and The number sut number Fraction	creatic trypsind b) were selecter of pfu's electer of pfu's electer (10 <sup>10</sup> pfu's) bound relative	inhibitor) is a controcted by binding to the tured after a binding end.  TABLE 9  and Semiconservative	ol and KKII/3 (5 and 6) and 6 and 6 and 6 target protesse, kallikrein. operiment as a fraction of the lay-phage, LACI-K1.	55	G H	aromatic Gly-only conformation "normal" conformation amphoteric aromatic aliphatic, branched β	nothing A, S, N, T Y, F, K, R	nothing  D, E, H, I, K, L, M, Q, R, V L, M, A, (C)  F, Y, W, G (C)			
ovine pane GI/3 (a and The number mumber fraction	creatic trypsin d b) were set ber of pfu's e e f (10 <sup>16</sup> pfu's) bound relativ	inhibitor) is a contracted by binding to the tuted after a binding etc.  to the parental displace to the parental displac	ol and KKII/3 (5 and 6) and target protease, kallikrein, operiment as a fraction of the lay-phage, LACI-K1.  The substitutions  Semi-conservative	55	G H I	aromatic Gly-only conformation "normal" conformation amphoteric aliphatic, branched β carbon	nothing A, S, N, T Y, F, K, R V, L, M, A	nothing  D, E, H, L, K, L, M, Q, R, V L, M, A, (C)  F, Y, W, G (C)  Q, N, S, T, D, E, A			
Initial	creatic trypsin d b) were sels ber of pfn's el r (10 <sup>10</sup> pfn's) bound relativ  Conservative  Category	inhibitor) is a controcted by binding to the tuted after a binding et a to the parental display to the	ol and KKII/3 (5 and 6) and 6 and 6 and 6 target protesse, kallikrein. operiment as a fraction of the lay-phage, LACI-K1.	60	G H I	aromatic Gly-only conformation 'normat' conformation amphoteric aromatic aliphatic, branched β carbon basic	nothing A, S, N, T Y, F, K, R V, L, M, A R, H	nothing  D, E, H, I, K, L, M, Q, R, V L, M, A, (C)  F, Y, W, G (C)  Q, N, S, T, D, E, A F, Y, W, H, (C)			
Direction of the state of the s	creatic trypsin d b) were set ber of pfu's e e f (10 <sup>16</sup> pfu's) bound relativ	inhibitor) is a controcted by binding to the tuted after a binding et a to the parental display to the	ol and KKII/3 (5 and 6) and target protease, kallikrein, operiment as a fraction of the lay-phage, LACI-K1.  The substitutions  Semi-conservative	55	G H I K L	aromatic Gly-only conformation 'normal' conformation amphoteric aromatic aliphatic, branched β carbon basic aliphatic	nothing A, S, N, T Y, F, K, R V, L, M, A R, H M, I, V, A	nothing  D, E, H, L, K, L, M, Q, R, V L, M, A, (C)  F, Y, W, G (C)  Q, N, S, T, D, E, A			

**TABLE 9-continued** 

### TABLE 15-continued

45 Under "Preferred", most highly preferred type are bold

	Conservative	e and Semiconserva	tive substitutions		Substitution to confer high affinity for pKA on KuDoms						
Initial AA type	Category	Conservative substitution	Semi-conservative substitution	5	Positio	n Preferred	Allowed	Unlikely to work			
P	hydrophilic inflexible	A, G, (E) V, I	A, (C), (D), (E), F, H, (K), L, M, N, Q, (R), S, T, W, Y	. 10	16	Ala, Gly	[Ser, Asp, Asn]	Pro. Thr, Val. Trp, Tyr) (Cys, Glu, Phe, His Ile, Lys, Leu, Met,			
Q	aliphatic plus amide	N, E, A, S, T, D	M, L, K, R	10	17	45- 4 0 W		Pro, Gla, Arg, Thr, Val, Trp, Tyr)			
R S	basic hydrophilic	K, Q, H A, T, G, N	S, T, E, D, A, D, E, R, K		17	Ala, Asn, Ser, Ee	Gly, Val, Glo, Thr	Cys, Asp. Phe, His, Pro, Arg, Tyr, (Glu, Lys, Met, Trp)			
T V	hydrophilic aliphatic, branched β carbon	A, S, G, N, V L, L, M, A, T	D, E, R, K, I P, (C)	15	18	His, Leu, Gln	(Ala,	Cys, Asp, Glu, Phe, Gly, Ile, Lys, Met, Asn, Pro, Arg, Ser,			
W Y	aromatic aromatic	F, Y, H F, W, H	L, M, I, V, (C) L, M, I, V, (C)		19	Pro, Gly, Leu	[Ast, He]	Thr, Val, Trp, Tyr Ala, Ghu, Gly, Met, Arg, Ser, Thr, Val, Trp, (Cys, Asp, Phe,			
semicons Changing	ervative if the from M to	he new cysteine E. R. K is semic	P. V. W. or Y to C is remains as a free thiol. conservative if the ionic	•	20	Arg	Leu, Ala, Ser, Lys, Gin, Val	His, Tyr) (Cys, Glu, Phe, Gly, His, Ile, Met, Asn, Pro, Thr, Trp,			
ip of the he methy	new side groups vlene groups	oup can reach the make hydroph	e protein surface while	25	21	Trp, Phe	[Tyr, His, Be]	Tyr) Cys, Leu (Ala, Asp, Glu, Gly, Lys, Met,			
		TO OF 12" IT" IT" O	TO SCHITCOHNELASHIAN					ASIL PTO, GILL ATO.			
f the side	e group is o	n or near the si	urface of the protein.		31	Gha	[Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile,	Asn, Pro, Gln, Arg, Ser, Thr, Val) (Arg, Lys, Cys, Phe, Gly, His, Met, Pro.			
f the side		n or near the su	urface of the protein.	30	31 32	Ghu Ghu, Gln	[Asp, Gin, Asn, Ser, Ala, Val, Leu, Ile, Tur] [Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly,	Ser, Thr, Val) (Arg, Lys, Cys, Phe, Gly, His, Met, Pro, Trp, Tyr) (Cys, Phe, His, Be,			
f the side	Definition of a 2 0123456789012	TABLE 14  Kunitz Domain (S  3  3445678901234567	urface of the protein.		_		Ala, Val, Leu, Ile, Thr) [Asp, Asn, Pro, Thr,	Ser, Thr, Val) (Arg, Lys, Cys, Phe, Gly, His, Met, Pro, Trp, Tyr) (Cys, Phe, His, Re, Lys, Met, Arg, Trp, Tyr) other 18 excluded. Cys, Asp, Glu, Phe, His, Lys, Met, Pro,			
234567890 XXXCXXXXX Vhere:	Definition of :  2 2123456789012 GENERAL XX  , X2, X3, X4, 11 = Phe, Tyr, T 2 = Tyr or Phe	TABLE 14  Kunitz Domain (S  3  2345678901234567  Kunux CxPxXXX  X58, X57, and X56	Harace of the protein.  EQ ID NO. 52)  4 5 /890123456789012345678 CEXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX		32 33 34 35 36	Glu, Gln Phe	Ab, Val, Leu, Ile, Thr  [Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly, Val]  [Tyr]  [Val, Ala, Asn, Gly, Leu]  [Trp, Phe]  Ser, Ala  (Other amino-acid types allowed only	Ser, Thr, Val) (Arg, Lys, Cys, Phe, Gly, His, Met, Pro, Thy, Tyr) (Cys, Phe, His, Ile, Lys, Met, Arg, Thp, Tyr) other 18 excluded. Cys, Asp, Glu, Phe, His, Lys, Met, Pro, Gln, Arg, Thp, Tyr (other 17)			
1234567890 1234567890 Where: X1 X2 X2 X3 X3 X4	Definition of :  2 2123456789012 GECERRERERER , X2, X3, X4, 1 = Phe, Tyr, T	TABLE 14  Kunitz Domain (S  3  34345678901234567  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	Harace of the protein.  EQ ID NO. 52)  4 5 /890123456789012345678 CEXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	30	32 33 34 35 36 37	Ghu, Gin  Phe Ser, Thr, He  Tyr Gly	Ab, Val, Leu, Ile, Thr] [Asp, Asn, Pro, Thr, Leu, Ser, Ala, Giy, Val] [Tyr] [Val, Ala, Asn, Gly, Leu] [Trp, Phe] Ser, Ala (Other amino-acid	Ser, Thr, Val) (Arg, Lys, Cys, Phe, Gly, His, Met, Pro, Trp, Tyr) (Cys, Phe, His, Re, Lys, Met, Arg, Trp, Tyr) other 18 excluded. Cys, Asp, Glu, Phe, His, Lys, Met, Pro, Gln, Arg, Trp, Tyr (other 17) (other 17)			

### TABLE 15

	Cubatination to			-	Under "Allowed" are types not actually tested, but judged to
	SHOPING OF COM	er high affinity for pK	A on KuDoms		be acceptable. Types shown in square brackets were allowed
Position	Preferred	Allowed	Unlikely to work	- 50	and not selected, but are so similar to types that were selected that the type is unlikely to abolish pKA binding.
10	Asp, Glu	Ala, Gly, Ser, Thr	Lys, Asn (Arg, Cys, Phe, His, Be, Leu, Met, Pro, Gh, Val,	- 30	Such types are not preferred, but pKA-binding proteins could have such types.
11	Asp, Gly, Ser, Val	Glu, Leu, Met, [Asn, Ile, Ala, Thr]	Trp, Tyr) (Cys, Phe, His, Lys, Pro, Gln, Arg, Trp, Tyr)	55	Under "Unlikely to work", types shown outside parentheses have been tried and no isolates had that type; types in parentheses have not been tested, but are judged to be
12	Gly	(Other amino acids ONLY if C <sub>14</sub> —C <sub>38</sub> disulfide replaced by other amino acids.)	23.5		unsuitable from consideration of the types actually excluded.
13	Arg, His, Pro, Asa,	(Thr, Ala, Gly, Lys,	Phe, Tyr, Cys, Leu,	60	TABLE 21
	Ser	Gin]	Ile, Val, Asp (Glu, Met, Trp)		First Variegation of LACI-K1
14	Cys	(Other amino acids ONLY if C <sub>38</sub> also changed.)	, 21 <i>p</i> )		a b 1 2 3 4 5 6 7 8 9 10 A E M H S F C A F K A D  igcelgagtatgleatitechtetigelgecitetaaglgetigati
15	Arg, Lys	[Ala, Ser, Gly, Met, Asn, Gln]	(Cys, Asp, Glu, Phe, His, Ile, Leu,	65	NsiI

TABLE 21-continued

### TABLE 21-continued

				TABLE 2	l-conti	inued	
First Variegation of LACI-K1				First Variegal	ion of L	ACI-KI	
I IN CIH FIS PIY YIC LIS LIS	5	C K	K MC	56 57 58 59 TRDG acticgtigacigg	i A		
LP WP WP  HIR QIR QIR  I IT MIT MIT  NIV KIV  LIP AID AE AE  D GHIR C K AIG G DIG G R  II 12 13 14 15 16 17 18 19 20  Igatiggth Nithgitaaaig Sinninnsinngkgti  F F P N I F T R Q C 21 22 23 24 25 26 27 28 29 30  Inchitchtchaaclatchtchacg kgtkag kgcl	15	The seguences variegation can g BstBI, or codon 42 priming of example, I	on gives 2 to in on a XbaI en and the ligonucles BstBI. To sequence	1.840 and fragment ads. Becau 3restriction otide, fill it tal variants than SEQ	a seque 32.768 having se of t a site, n, and a are 2.7	variants. 'Mul and the closen one will cut with N/26×10° at 2.56.	55.536 DN, ond group of This variega one of Agel ess between make a self fluI and, for ad 8.59×10°
QEE NIH MIK CII FIS FIY	25	The amino	acid seq	uence has TABL		D NO. 57.	
YIC LIS LIP WIP				Specificity	Results		
HIR QIR	30	Protein displayed		-		yme tested	
NIV KIV ALD ALE EKQEKQFGWYGGCGEDGNQ		ou M13	Plasmin	Thrombin	pKA	Trypsin	Trypsin, 2 washes
SagiSaaktriNNShackgriggthgthNSkgribackagi	35	LACI-KI KkII/3(D) BPTI	1 3.4 88.	1 1.5 1.1	1 196. 1.7	1 . 2. 0.3	1 1.4 .8

N R F E S L E E 43 44 45 46 47 48 49 50 laackgghte (gaaletictalgagigaal i BstBI i/XbaI i I AgeI I

Numbers refer to relative binding of phage display clones compared to the parental phage display.

The KkII/3(D)(Kallikrein) clone retains the parental molecule's affinity for trypsin. KkII/3(D) was selected for binding to pKA.

### TABLE 24

IA	BLE 24								
Mat a S. cerevisiae expression vectors:									
Matox1 (Mfo8)  K R P R  5'IAAAIAGGICCTICGAIG3'    Stull     Xhol	SEQ ID NO. 58 SEQ ID NO. 59								
Mator2 (after introduction of a linker into Stul	-cut DNA)								

2 (after introduction of a linker into Stul-cut DNA)

v	Ð	ъ			_			a	mino:	acids:	SEQ I	D NO.	. 60	
- A	т.	E	A	A	E	P	W	_	_			_		
5'IAAA	IAGG	<b>IGAA</b>	<b>IGCG</b>	GCC	GAC	HOCA	ma	NO.	ICCC	***		~~~	E	
			1.1	Root				~~~		II MA	JIAGI	CICIC	AGO'	
				-oki	<u>-</u> '	- 30	YI	<u> </u>	Kasi	_!	1	Xho	I I	
													NO. 61	

Mator-LACI-K1, amino acids: SEQ ID NO. 62, DNA: SEQ ID NO. 63

a b c d 1 2 3 4 5 6 7 8

K R E A A E M H S F C A F K

5'IAAAIAGGIGAAKGCGIQCCKGAGlatgicaticciticcitgclgctittclaaal

i Eagl i Nsii !

TABLE 24-continued

Mat a S. cerevisine expression vectors: 9 10 11 12 13 14 15 16 17 18 19 20 A D D G P C K A I M K R lgctlgatlgaClggTccGttgtlaaalgctlatclatglbaalcgtl | RsrII | (BspHIII 21 22 23 24 25 26 27 28 29 30 FFFNIFT R Q C htelttelttelaaelattittelaeGk gt leaghgel 1 Mhul 1 31 32 33 3435 36 37 38 39 40 41 42 E E F I Y G G C E G N Q lgaglgaAltiClatittaclggtlggtltgtlgaalggt haclcagl | EcoRI | | BstEII | 43 44 45 46 4748 49 50 N R F E S L E E laacicgGlittelgaaltetletAlgagigaal | AgeI | 51 52 53 54 55 56 57 58 59 60 C K K M C T R D G A hgttaaglaaglatghgclacticgtigaciggclgcclTAAITACHCTCIGAGI-3'

| Kasl | | Xhol |

We expect that Mat $\alpha$  pre sequence is cleaved before  $GLU_a$ — $ALA_b$ —

### TABLE 27

High specificity plasma Kallikrein inhibitors
LACI-KI (SEQ ID NO. 3)  MHSPCAFKADDGPCKAIMKRFFFNIFTRQCEEFTYGGCEGNQNRFESLEECKKMCTRD  KKII/3/7 (SEQ ID NO. 11)  mhsfcaftaddgHckANHQrffiniftrqcEEfSyggcGgnqnrfesleeckkmctrd  KKII/3/7-K15A (SEQ ID NO. 64)  mhsfcaftaddghcAanhqrffiniftrqceefsyggcggnqnrfesleeckkmctrd  KK2/#13-R15A (SEQ ID NO. 65)  mhsfcaftaDGgRcAGAHPrWffniftrqcEEfSyggcGgnqnrfesleeckkmctrd  KK2/#11-R15S (SEQ ID NO. 66)  mhsfcafkaddgpcSaahprwffniftrqceefsyggcggnqnrfesleeckkmctrd

### TABLE 49

		Residue Number										
	10	11	12	13	14	15	16	17	18	19	20	21
LACI-K1 Consensus of KKII/3 selectants	D d	D d	G g	P H	C	K	A	I N	M H	K Q	R	F f
KK Library #2  KK2/#13	NK DE	NI AD ST GV	g	FS YC LP HR IT NV AD G	c	KR	AG	NI AD ST GV	QL HP R	QL HIP R	r	FW CL
KK2/#14	_	G	_		-	_	_		_	_	_	_
KK2/#5		_	_	_	_	_	_	_		_	_	_
KK2/#11	_	_	_	P	_		A	_	_			
KK2/#1	_	v		P	_	_	A		_	-	_	_
KK2/#4	_	v	_	_		_	_			_	_	_
KK2/#6				s	_	_	_	_	Q	_	-	F
KK2/#10	E	G		S	_	_	A	_		L		_
	~	•	_	3	_		A	_	-	Q	_	

TABLE 49-continued

		Residue Number											
	01	11	12	13	14	15	16	17	18	19	20	21	
KK2/#8				P		_	_			ī		F	
KK2/#3	_		_	H	_	_			1			_	
KK2/#9	_	S	_	N	_	_	_	N	ĩ	_	_	F	
KK2/#7		S		_	_		_	N		0	_	F	
KK2/#12	_	G				_	Α	Ţ	0			-	
Consensus #2	D	D	g	R	c	R	G	Ā	H	P	r	W	

#### TABLE 750

DNA that embodies Library KKF. A F 1 2 3 4 5 6 7 8 5'-ceteet atg cat tee tie tge gee tie aag get RaS I Nsi FIS YIC LOH RII IN VIT VIT (M) (K) TIA AIG AIS LIP LIP SIG DIN GED QIR RIH DIN G P C KIR ANG I IN H 11 12 13 14 15 16 17 18 RNt ggt NNt tgt aRa gSt RNt cNS cNS cgt FIC F F N I F T R (SEQIDNO. 69) 22 23 24 25 26 27 28 tKS the the age ate the agg egt teeetee-3" (SEQ ID NO. 67) 3'-g fig tag aag tgc gca agggagg-5' (SEQ ID NO. 68) | MluI |

The RsrII and BspHI sites found in the parental LACI-K1 display gene (Table 6) are not present in Library KKF.

There are 1,536,000 amino-acid sequences and 4,194,304 DNA sequences. Met, and Lys19 are not allowed in Library KKF.

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   119-26.
                                                            23:2108-2:112.
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                                                         WUNT88: Wun et al., J. BIOL. CHEM. (1988)
 VARA83: Varadi & Patthy. Biochemistry (1983)
                                                            263:6001-6004.
   22:2440-2446.
                                                SEQUENCE LISTING
   ( 1 ) GENERAL INFORMATION:
       ( i i i ) NUMBER OF SEQUENCES: 70
   ( 2 ) INFORMATION FOR SEQ ID NO:1:
          ( i ) SEQUENCE CHARACTERISTICS:
                  ( A ) LENGTH:8 arrino acids
                  (B) TYPE: amino acid
                 ( C ) STRANDEDNESS: single
                 ( D ) TOPOLOGY: linear
        ( i i ) MOLECULE TYPE: protein
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
  lle Val Gly Gly Thr Asn Ser Ser
  (2) INFORMATION FOR SEQ ID NO:2:
         ( i ) SEQUENCE CHARACTERISTICS:
                 ( A ) LENGTH:58 amino acids
                 (B) TYPE: amino acid
                 (C) STRANDEDNESS: single
                ( D ) TOPOLOGY: Imeas
       ( i i ) MOLECULE TYPE: protein
       ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:2:
 Arg lie lie Arg Tyr Phe Tyr Asa Ala Lys Ala Gly Leu Cys Gin Thr
      Val Tyr Gly Gly Cya Arg Ala Lys Arg Asn Asn Phe Lys Ser Ala
 Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
 (2) INFORMATION FOR SEQ ID NO:3:
        ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:58 amino acids
               ( B ) TYPE: amino acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: hnear
      ( i i ) MOLECULE TYPE: protein
      ( x i ) SEQUENCE DESCRIPTION; SEQ ID NO3:
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala
lie Met Lys Arg Phe Phe Phe Asa lle Phe Thr Arg Gin Cys Giu Glu
Phe Ile Tyr Gly Gly Cys Glu Gly Asa Gla Asa Arg Pho Glu Ser Lea
```

```
-continued
```

```
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
(2) INFORMATION FOR SEQ ID NO:4:
       ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:58 amino acids
               ( B ) TYPE: amino acid
```

### ( i i ) MOLECULE TYPE: protein

### ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:4:

( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 1 10 15 His Gla Arg Phe Phe Phe Asa Ile Phe Thr Arg Gla Cys Glu Glu
20 25 · 30 Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Glu Ser Leu
35 40 45

### ( 2 ) INFORMATION FOR SEQ ID NO.5:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid .
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

### ( $\mathbf{x}$ $\mathbf{i}$ ) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
5 10 15 Ser Leu Pro Arg Phe Phe Phe Asa Ile Phe Thr Arg Gla Cys Glu Glu 20 25 30

### (2) INFORMATION FOR SEQ ID NO.6:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: autino acid ( D ) TOPOLOGY: Emean
  - (C) STRANDEDNESS: single

### ( i i ) MOLECULE TYPE: protein

### ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala 1 5 10 15 His Leu Arg Phe Phe Phe Asa Ile Phe Thr Arg Gla Cys Glu Glu 20 25 30 Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Gla Ser Lea 35 40 45 Glu Cys Lys Lys Met Cys Thr Arg Asp
50
55

### (2) INFORMATION FOR SEQ ID NO:7:

```
( i ) SEQUENCE CHARACTERISTICS:
```

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

#### ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 1 5 10 15

Asn His Gla Arg Phe Phe Phe Asa lle Phe Thr Arg Gla Cys Glu Gla 20

Phe Thr Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glo Ser Lev 35

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50
55

### ( 2 ) INFORMATION FOR SEQ ID NO:8:

### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

### (x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 5 10 15

Asn His Gla Arg Phe Phe Asa lle Phe Thr Arg Gla Cys Glu Gla 20 25 30

Phe Thr Tyr Gly Gly Cys Ala Gly Asn Gla Asn Arg Phe Gla Ser Lea 35 40 45

Glu Clu Cys Lys Lys Met Cys Thr Arg Asp
50
55

### (2) INFORMATION FOR SEQ ID NO:9:

### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- (D) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

### ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 1 5 10 15

Ser Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30

Phe lle Tyr Gly Gly Cys Gly Gly Asn Gla Asn Arg Phe Glu Ser Lev 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50
55

### ( 2 ) INFORMATION FOR SEQ ID NO:10:

### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single

#### ( D ) TOPOLOGY: finear

- ( i i ) MOLECULE TYPE: protein
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 1015

Asn His Gla Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu. 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glo Ser Leu 35 40 45

Glu Glu Cys Lys Lys Met Cys The Arg Asp 50 55

- (2) INFORMATION FOR SEQ ID NO:11:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - ( B ) TYPE: amino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala 1 5 10 15

Asa His Gla Arg Phe Phe Phe Asa Ile Phe Thr Arg Gla Cys Glu Glu 20 25

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glo Ser Leo 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp

- (2) INFORMATION FOR SBQ ID NO:12:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - (B) TYPE: amino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - (x i ) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala I 10 15

Asa His Glu Arg Phe Phe Phe Asa Ile Phe Thr Arg Glu Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp 50 55

- (2) INFORMATION FOR SEQ ID NO:13:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - ( B ) TYPE: amino acid
    - ( C ) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - ( i i ) MOLBCULE TYPE: protein
  - ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
      Met His Ser Phe Cys Ata Phe Lys Ala Asp 10
      Asp Gly His Cys Lys Ala 15

      Asn His Gla Arg Phe Phe Phe Asn Ile 20
      Phe Phe Phe Asn Ile 25

      Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gla Asn Arg Phe Glu Ser Leu 35

      Glu Glu Cys Lys Lys Met Cys Thr Arg Asp 50
```

### (2) INFORMATION FOR SEQ ID NO:14:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
      Met His Ser Phe Cys Ala Phe Lys Ala Asp 10
      Asp Gly His Cys Lys Gly 10

      Ala His Leu Arg 20
      Phe Phe Phe Asa Jle 25
      Phe Thr Arg Gln Cys Glu Glo 30

      Phe Ile Tyr Gly Gly Cys Glu Gly Asa Gla Asa Arg Phe Glu Ser Leu 50
      Thr Arg Asp She Glu Glu Ser Leu 50
```

### ( 2 ) INFORMATION FOR SEQ ID NO:15:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{x}$  i ) SEQUENCE DESCRIPTION: SEQ ID NO:15:

### ( 2 ) INFORMATION FOR SEQ ID NO:16:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Mer His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala
1 5 10 15

Ilo His Leu Arg Phe Phe Phe Asm Ile Phe Thr Arg Glm Cys Glu Glo

Phe Ile Tyr Gly Gly Cys Glu Gly Ash Gla Ash Arg Phe Glu Ser Leu
35
40
Glu Glu Cys Lys Lys Mer Cys Thr Arg Asp
50
55

### (2) INFORMATION FOR SEQ ID NO:17:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH-58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

### ( $\mathbf{x}$ i ) SEQUENCE DESCRIPTION: SEQ ID NO:17:

### ( 2 ) INFORMATION FOR SEQ ID NO:18:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH: 304 amino acids
  - (B) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

### ( $\times$ i ) SEQUENCE DESCRIPTION: SEQ ID NO:18:

			180												
								185					190		
							Pro 200					205			
											220				
							Arg			233					240
							Ser		230					255	
							Leu	205					270		
							Leu 280					285			
Lys	G1 n 290	Arg	V a 1	Lys	1 1 e	Ala 295	Tyr	Glu	GlB	i 1 e	Phc 300	V a 1	Lys	Азв	Met

#### ( 2 ) INFORMATION FOR SEQ ID NO:19:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

### ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:19:

 Met
 His
 Ser
 Phe
 Cys
 Ala
 Phe
 Lys
 Ala
 Asp
 Gly
 Gly
 Arg
 Cys
 Arg
 Gly
 Gly
 Gly
 Gly
 Arg
 Cys
 Arg
 Gly
 Asp
 Gly
 Asp
 Gly
 Gly
 Gly
 Gly
 Asp
 Gly
 Asp
 Fig
 Gly
 Gly
 Gly
 Gly
 Asp
 Fig
 Gly
 Gly
 Gly
 Gly
 Gly
 Asp
 Fig
 Gly
 G

#### (2) INFORMATION FOR SEQ ID NO:20:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- (D) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

#### ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Mot His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly 1 10 15

Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gln Cys Glu Glu 20 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Gln Ser Leu
35
40
45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp 50 55

#### (2) INFORMATION FOR SEQ ID NO:21:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single

```
(D) TOPOLOGY: linear
```

- ( i i ) MOLECULE TYPE: protein
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala 1 5 10 15

Ala His Pro Arg Trp Phe Phe Ara Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gla Asn Arg Phe Glu Ser Lev 35 40 45

Giu Giu Cys Lys Lys Met Cys The Arg Asp 50 55.

- ( 2 ) INFORMATION FOR SEQ ID NO:22:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - (B) TYPE: amino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: fincar
  - ( i i ) MOLECULE TYPE: protein
  - ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO.22:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala I 5 10 15

Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gln Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Gla Ser Lea 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Atg Asp
50
55

- (2) INFORMATION FOR SEQ ID NO:23:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - (B) TYPE: amino acid
    - ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Mot His Scr Pho Cys Ala Pho Lys Ala Asp Val Gly Arg Cys Arg Gly
10 15

Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gla Cys Glu Glu 20 25 30

Phe Sor Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Gln Ser Lou 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Azg Asp
50
55

- (2) INFORMATION FOR SEQ ID NO:24:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENOTH:58 amino acids
    - ( B ) TYPE: amino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:24:

#### (2) INFORMATION FOR SEQ ID NO:25:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- (  $\mathbf{x}$  i ) SEQUENCE DESCRIPTION: SEQ ID NO:25:

 Met His Ser Phe Cys Ala Phe Lys Ala Asp 10
 Asp Gly Ser Cys Arg Ala Asp 15

 Ala His Leu Arg 20
 Trp Phe Phe Phe Asn 11e 25
 Phe Thr Arg Gln Cys Glu 30
 Gln Cys Glu Glu 45

 Phe Ser Tyr Gly Gly Cys Gly 40
 Gly Gly Asn Gln Asn Arg 45
 Phe Gln Ser Leu 45

Gin Gin Cys Lys Lys Met Cys Thr Ar. 8 As a

#### ( 2 ) INFORMATION FOR SEQ ID NO:26:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{z}$   $\mathbf{i}$  ) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Met His Ser Phe Cys Ala Phe Lys Ala Glu Gly Gly Ser Cys Arg Ala 1 5 10 15

Ala Bis Gln Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Glu Ser Leu 35 40 45

Glu Glu Cya Lya Lya Met Cya The Arg Asp
50
55

#### ( 2 ) INFORMATION FOR SEQ ID NO:27:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- (  $\mathbf{z}$   $\mathbf{i}$  ) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Gly
1 5 10 15

Ala His Lev Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu

```
-continued
```

20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45

Glu Glu Cys Lys Lys Mer Cys Thr Arg Asp
50 55

#### (2) INFORMATION FOR SEQ 1D NO:28:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:28:

#### (2) INFORMATION FOR SEQ ID NO:29:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{x}$  i ) SEQUENCE DESCRIPTION: SEQ ID NO:29:

 Met
 His
 Ser
 Phe
 Cys
 Ala
 Phe
 Lys
 Ala
 Asp
 Ser
 Gly
 Asa
 Cys
 Arg
 Gly
 Arg
 Gly
 Asa
 Cys
 Arg
 Gly
 Gly
 Gly
 Gly
 Gly
 Gly
 Gly
 Gly
 Gly
 Asa
 Gly
 Thr
 Arg
 Gly
 Gly
 Gly
 Gly
 Asa
 Gln
 Asa
 Cys
 Gly
 Gly
 Gly
 Asa
 Gln
 Asa
 Arg
 Gly
 Gly
 Gly
 Gly
 Asa
 Gln
 Asa
 Arg
 Gly
 Gly
 Gly
 Gly
 Asa
 Gly
 Asa
 Cys
 Lys
 Lys
 Asa
 Asa
 Gly
 Asa
 Cys
 Asa
 Cys
 Gly
 Gly
 Gly
 Asa
 Gly
 Arg
 Gly
 Gly
 Gly
 Asa
 Gly
 Asa
 Cys
 Arg
 Gly
 Gly
 Arg
 Gly
 Arg
 Gly
 Arg
 Arg
 Gly
 Arg
 Gly
 Arg
 Arg
 Arg
 Arg
 Gly
 Arg
 Arg
 A

#### (2) INFORMATION FOR SEQ ID NO:30:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 agains acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{x}$  i ) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Mer His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Arg Cys Arg Gly L5

Ash His Gla Arg Phe Phe Phe Ash lle Phe Thr Arg Gln Cys Glu Glu 25

Phe Ser Tyr Gly Gly Cys Gly Gly Ash Gln Ash Arg Phe Glu Ser Leu 45

```
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
```

#### ( 2 ) INFORMATION FOR SEQ ID NO:31:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein

### (x i ) SEQUENCE DESCRIPTION: SEQ ID NO-31:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Ala t 5 10 15

Ile Gla Pro Arg Trp Phe Phe Asa Ile Phe Thr Arg Gla Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35
40
45

Glu Glo Cys Lys Lys Met Cys Thr Arg Asg
50
55

#### (2) INFORMATION FOR SEQ ID NO:32:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: action acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

### (x i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Arg Gly I 5 10 15

Ala His Pro Arg Trp Phe Phe Assa Ile Phe Thr Arg Gla Cya Glu Glu 25

Phe Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Glu Ser Leu 35 40 45

Glu Glu Cys Lys Lys Met Cys The Arg Asp 50 55

#### (2) INFORMATION FOR SEQ ID NO:33:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- (B) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- (D) TOPOLOGY: finear

#### ( i i ) MOLECULE TYPE: protein

#### ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO.33:

Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Pro Gly Ite Cys Arg Gly
1 5 10 15

Tyr Ile Thr Arg Tyr Pho Tyr Asa Asa Gla Thr Lys Gla Cys Gla Arg 20 25 30

Phe Lys Tyr Gly Gly Cys Lou Gly Asa Mot Asa Asa Phe Glu Thr Leu 35 40 45

Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly
50 55

#### (2) INFORMATION FOR SEQ ID NO:34:

```
( i ) SEQUENCE CHARACTERISTICS:
                 ( A ) LENGTH:58 amino acids
                 ( B ) TYPE: amino acid
                 ( C ) STRANDEDNESS: single
                 ( D ) TOPOLOGY: finear
        ( i i ) MOLECULE TYPE: protein
        ( \mathbf{x} \mathbf{i} ) SEQUENCE DESCRIPTION: SEQ ID NO:34:
 Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Gly Gly Arg Cys Arg Gly
1 5 10 15
       His Pro Arg Trp Phe Tyr Asn Asn Gin Thr Lys Gln Cys Glu Glu 20 25 30
       Ser Tyr Gly Gly Cys Giy Gly Asn Met Asa Asn Phe Glu Thr Leu
35
40
45
       Glu Cys Lys Asa Ile Cys Glu Asp Gly 50
 (2) INFORMATION FOR SEQ ID NO:35:
        ( i ) SEQUENCE CHARACTERISTICS:
                ( A ) LENGTH:58 amino acids
                ( B ) TYPE: amino acid
                ( C ) STRANDEDNESS: single
                ( D ) TOPOLOGY: finest
       ( i i ) MOLECULE TYPE: protein
       ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:35:
 Gly Pro Ser Trp Cys Leu Thr Pro Ala Asp Arg Gly Leu Cys Arg Ala
1 5. 10 15
Ash Glu Ash Arg Phe Tyr Tyr Ash Ser Val He Gly Lys Cys Arg Pro
20 25 30
Phe Lys Tyr Ser Gly Cys Gly Gly Asn Glu Asn Asn Phe Thr Ser Lys
35
40
45
Gla Glu Cys Leu Arg Ala Cys Lys Lys Gly
50
55
(2) INFORMATION FOR SEQ ID NO:36:
       ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENOTH:58 amino acids
               ( B ) TYPE: amino acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: Knew
     ( i i ) MOLECULE TYPE: protein
     ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:36:
           Ser Trp Cys Leu Thr Pro Ala Asp Asp Gly Pro Cys Arg Ala
5 10 15
Ala His Pro Arg Phe Tyr Tyr Asa Ser Val He Gly Lys Cys Glu Pro
20 25 30
    Ser Tyr Ser Gly Cys Gly Gly Asa Glo Asa Asa Phe Thr Ser Lys
```

#### (2) INFORMATION FOR SEQ ID NO:37:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single

```
( D ) TOPOLOGY: linear
```

- ( i i ) MOLECULE TYPE: protein
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:37:
- Glu Thr Asp lle Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Arg Asp 1 5 10
- Phe Ile Leu Lys Trp Tyr Tyr Asp Pro Asa Thr Lys Ser Cys Ala Arg 20 25 30
- Phe Trp Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly Ser Gln 35 40 45
- Lys Glu Cys Glu Lys Val Cys Ala Pro Val 50 55
- (2) INFORMATION FOR SEQ ID NO:38:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - ( B ) TYPE: autino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:38:
- Glu Thr Asp lie Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Arg Ala 1 5 10 15
- Ala His Leu Lys Trp Tyr Tyr Asp Pro Asn Thr Lys Ser Cys Ala Glu 20 25 30
- Phe Sor Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly Ser Gla
  35
  40
  45
- Lys Gin Cys Glu Lys Val Cys Ala Pro Val 50 55
- (2) INFORMATION FOR SEQ ID NO:39:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - ( B ) TYPE: amino acid
    - ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear
  - ( i i ) MOLECULE TYPE: protein
  - ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:39:
- Asa Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys Arg Ala 1 5 10 15
- Lev Lev Lev Arg Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys Arg Gla
  26 25 30
- Phe Lee Tyr Gly Gly Cys Gle Gly Asn Ala Asn Asn Phe Tyr Thr Trp
  35 40 45
- Glu Ala Cys Asp Asp Ala Cys Trp Arg 11e
- (2) INFORMATION FOR SEQ ID NO:40:
  - ( i ) SEQUENCE CHARACTERISTICS:
    - ( A ) LENGTH:58 amino acids
    - ( B ) TYPE: autino acid
    - ( C ) STRANDEDNESS: single
    - ( D ) TOPOLOGY: finest
  - ( i i ) MOLECULE TYPE: protein
  - (x i) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Asn Ala Glu I ie Cys Leu Leu Pro Leu Asp Gly Gly Pro Cys Arg Ala

Ala His Leu Arg Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys Glu Gln

20

Phe Ser Tyr Gly Gly Cys Glu Gly Asn Ala Asn Asn Phe Tyr Thr Trp

35

Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile

#### (2) INFORMATION FOR SEQ ID NO:41:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:61 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: finear

#### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{x} \ \mathbf{i} \ )$  SEQUENCE DESCRIPTION: SEQ ID NO:41:

 Val
 Pro
 Lys
 Val
 Cys
 Arg
 Leu
 Gln
 Val
 Ser
 Val
 Asp
 Asp
 Gln
 Cys
 Glu

 Gly
 Ser
 Thr
 Glu
 Lys
 Tyr
 Phe
 Phe
 Asn
 Leu
 Ser
 Ser
 Met
 Thr
 Cys
 Glu

 Lys
 Phe
 Phe
 Phe
 Asn
 Arg
 Asn
 Arg
 Ile
 Glu
 Asn
 Arg
 Phe

 Pro
 Asp
 Glu
 Ala
 Thr
 Cys
 Met
 Gly
 Phe
 Cys
 Ala
 Pro
 Lys

#### ( $^2$ ) INFORMATION FOR SEQ ID NO:42:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:61 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

(  $\pi\ i$  ) SEQUENCE DESCRIPTION: SEQ ID NO:42:

 Val
 Pro
 Lys
 Val
 Cys
 Arg
 Leu
 Oln
 Val
 Ser
 Val
 Asp
 Asp
 Gla
 Cys
 Arg

 Ala
 Ala
 His
 Pro
 Lys
 Tyr
 Phe
 Phe
 Asn
 Leu
 Ser
 Ser
 Met
 Thr
 Cys
 Glu

 Glu
 Phe
 Phe
 Phe
 Asa
 Arg
 Arg
 Ile
 Glu
 Asa
 Arg
 Phe
 Asa
 Arg
 Ile
 Glu
 Asa
 Arg
 Phe
 Phe
 Asa
 Arg
 Ile
 Glu
 Asa
 Arg
 Phe
 Phe
 Asa
 Arg
 Ile
 Glu
 Asa
 Arg
 Phe
 Phe
 Asa
 Arg
 Ile
 Asa
 Arg
 Phe
 Phe
 Asa
 Arg
 Ile
 Arg
 Phe
 P

#### (2) INFORMATION FOR SEQ ID NO:43:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amiso acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ile Pro Ser Phe Cys Tyr Ser Pro Lys Asp Glu Gly Leu Cys Ser Ala 10 15

Asp Val Thr Arg Tyr Tyr Phe Asp Pro Arg Tyr Arg Thr Cys Asp Ala

Phe Thr Tyr Thr Gly Cys Gly Gly Asn Asp Asn Asn Phe Val Ser Arg

Glu Asp Cys Lys Arg Ala Cys Ala Lys Ala

50

#### (2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:44:

 I le
 Pro
 Ser
 Phe
 Cys
 Tyr
 Ser
 Pro
 Lys
 Asp
 Glu
 Gly
 His
 Cys
 Arg
 Ala

 1
 5
 10
 10
 15

Ala
His Gla Arg Tyr
 Tyr
 Pro
 Arg
 Tyr
 Arg
 Thr
 Cys
 Asp
 Ala

 20
 25
 25
 30

25 30
Phe Thr Tyr Thr Gly Cys Gly Gly Asa Asp Ash Ash Phe Val Ser Arg
35 40 45

Glu Asp Cys Lys Arg Ala Cys Ala Lys Ala 50 55

#### ( 2 ) INFORMATION FOR SBQ ID NO:45:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid (C) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Lys Glu Asp Ser Cys Glu Leu Gly Tyr Ser Ala Gly Pro Cys Met Gly I 5 10 15

Met Thr Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Glu Thr 20 25 30

Phe Gln Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45 .

Lys Gin Cys Lon Gin Thr Cys Arg Thr Val

#### (2) INFORMATION FOR SEQ ID NO:46:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single (D) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- . . .
- (  $\mathbf{x} \ \mathbf{i} \ )$  SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Glu Asp Ser Cys Gln Leu Gly Tyr Asp Ala Gly Pro Cys Arg Gly
1 10 15

Ala His Pro Arg Tyr Phe Tyr Asa Gly Thr Ser Met Ala Cys Glu Thr 20 25

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gly Asn Asn Phe Val Thr Glu 35

```
-continued
```

```
Lys Giu Cys Leu Gla Thr Cys Arg Thr Val
  (2) INFORMATION FOR SEQ ID NO:47:
         ( i ) SEQUENCE CHARACTERISTICS:
                ( A ) LENGTH:58 amino acids
                ( B ) TYPE: amino acid
                ( C ) STRANDEDNESS: single
                ( D ) TOPOLOGY: linear
       ( i i ) MOLECULE TYPE: protein
       ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:47:
 Thr Val Ala Ala Cys Asa Leu Pro Ile Val Arg Gly Pro Cys Arg Ala
1 5 15
      lle Gin Leu Trp Ala Phe Asp Ala Vai Lys Gly Lys Cys Vai Leu
20 25 30
       Pro Tyr Gly Gly Cys Gln Gly Asa Gly Asa Lys Phe Tyr Ser Glu
35 40 45
 Lys Glu Cys Arg Glu Tyr Cys Gly Val Pro
 (2) INFORMATION FOR SEQ ID NO:48:
        ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:58 amino acids
                (B) TYPE: amino acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: finest
       ( i i ) MOLECULE TYPE: protein
       ( \mathbf{z} \ \mathbf{i} ) SEQUENCE DESCRIPTION: SEQ ID NO:48:
 The Val Ala Ala Cys Asa Leu Pro Ile Asp Asp Gly Pro Cys Arg Ala
1 5 10 15
     His Gla Leu Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Glu Glu 20 25 30
Phe Ser Tyr Gly Gly Cys Glu Gly Asa Gly Asa Lys Phe Tyr Ser Glu 35 40 45
     Glu Cys Arg Glu Tyr Cys Gly Vai Pro-
50 55
 (2) INFORMATION FOR SEQ ID NO:49:
        ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:58 amino acids
               ( B ) TYPE: smino acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE: protein
      (x i ) SEQUENCE DESCRIPTION: SEQ ID NO:49:
Thr Val Ala Ala Cys Asa Leu Pro 11e Asp Asp Gly Pro Cys Arg Ala
1 5 10 15
Ala His Gla Arg Trp Ala Phe Asp Ala Val Lys Gly Lyr Cys Glu Glo
20 25 30
     Ser Tyr Gly Gly Cys Glo Gly Asn Gly Asn Lys Pho Tyr Ser Glo 35 40 45
Lys Glu Cys Arg Glu Tyr Cys Gly Val Pro
```

```
( i ) SEQUENCE CHARACTERISTICS:
```

- ( A ) LENGTH:58 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

#### ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Val Arg Glu Val Cys Ser Glu Gla Ala Glu Thr Gly Pro Cys Arg Ala 1 5 10 15

Met Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro 20 25 30

Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu 35

Glu Tyr Cys Met Ala Val Cys Gly Set Ala 50 55

#### ( 2 ) INFORMATION FOR SEQ ID NO.51:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- ( B ) TYPE: amino acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: finear

#### ( i i ) MOLECULE TYPE: protein

#### ( $\mathbf{z}_{-}\mathbf{i}_{-}$ ) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Val Arg Glu Val Cys Ser Glu Gln Ata Glu Thr Gly Pro Cys Arg Ala 1 5 10 15

Ala His Pro Arg Trp Tyr Pho Asp Val Thr Glu Gly Lys Cys Glu Glu 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Ann Arg Asn Asn Phe Asp Thr Glo
45

Glu Tyr Cys Met Ala Val Cys Gly Ser Ala

#### (2) INFORMATION FOR SEQ ID NO:52:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- ( B ) TYPE: amino acid.
- ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

#### ( $\mathbf{x} \cdot \mathbf{i}$ ) SEQUENCE DESCRIPTION: SBQ ID NO:52:

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Gly Xaa Cys Xaa Xaa I

Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa 50 55

#### ( 2 ) INFORMATION FOR SEQ ID NO.53:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH:58 amino acids
- ( B ) TYPE: amino acid
- (C) STRANDEDNESS: single

```
( D ) TOPOLOGY: linear
```

#### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu Pro Asn Val Cys Ala Phe Pro Met Glu Lys Gly Pro Cys Glu Thr
1 5 10 15

Tyr Met Thr Arg Trp Phe Phe Asa Phe Glu Thr Gly Glu Cys Glu Leu 20 25 30

Phe Ala Tyr Gly Gly Cys Gly Gly Asn Ser Asn Asa Phe Leo Arg Lys 35 40 45

GIu Lys Cys Glu Lys Phe Cys Lys Phe Thr 50 55

#### (2) INFORMATION FOR SEQ ID NO.54:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

(  $\mathbf{x}\ \mathbf{i}\ )$  SEQUENCE DESCRIPTION: SEQ ID NO.54:

Leu Pro Asu Val Cys Ala Phe Pro Met Glu Asp Gly Pro Cys Arg Ala 1 5 10 15

Ala His Pro Arg Trp Phe Phe Asa Phe Glu Thr Gly Glu Cys Glu Glu 20 25 30

Pho Ala Tyr Gly Gly Cys Gly Gly Asa Ser Asa Asa Phe Leu Arg Lys
35
40
45

Glo Lys Cys Glu Lys Phe Cys Lys Phe Thr
50
55

#### ( 2 ) INFORMATION FOR SEQ ID NO:55:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:38 arriso acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Arg Pro Asp Phe Cys Leu Glu Pro Pro Glu Thr Gly Pro Cys Arg Ala 1 10 15

Ala His Pro Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Glu Glu 20 25 30

Phe Val Tyr Gly Gly Cys Gly Ala Lys Arg Asn Asn Phe Lys Ser Ala
35
40
45

Gln Asp Cys Met Arg Thr Cys Gly Gly Ala 50 55

#### (2) INFORMATION FOR SEQ ID NO:56:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 186 bases
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE:other nucleic acid

( A ) DESCRIPTION: synthetic DNA fragment

```
-continued
         ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:56:
  GCCGAGATGC ATTCCTTCTG CGCCTTCAAG GCTGATGATG GTCNTTGTAA
                                                                                          5 0
  AGSTNNTNNS NNGCGTTTCT TCTTCAACAT CITCACGCGI CAGTGCSAGS
                                                                                        100
  AATICNNSTA CGGTGGTTGT NNSGGTAACC AGAACCGGTT CGAATCTCTA
                                                                                        150
  GAGGAATGTA AGAAGATGTG CACTCGTGAC GGCGCC
                                                                                        186
  (2) INFORMATION FOR SEQ ID NO:57:
         ( i ) SEQUENCE CHARACTERISTICS:
                 ( A ) LENGTH:62 amino acids
                 ( B ) TYPE: amino acid
                 ( C ) STRANDEDNESS: single
                 ( D ) TOPOLOGY: linear
        ( i i ) MOLECULE TYPE: protein
        ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:57:
 Ala Glu Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Xaa Cys
 Xaa Xaa Phe Xaa Tyr Gly Gly Cys Xaa Gly Asn Gln Asn Arg Phe Gin
35 40 45
 Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp Gly Ala
 (2) INFORMATION FOR SEQ ID NO:58:
        ( i ) SEQUENCE CHARACTERISTICS:
                ( A ) LENGTH's amino acids
                ( B ) TYPE: amino acid
                ( C ) STRANDEDNESS: single
                (D) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE: protein
      (x i) SEQUENCE DESCRIPTION: SEQ ID NO.58:
(2) INFORMATION FOR SEQ ID NO:59:
        ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH: 13 bases
               ( B ) TYPE: aucleic acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE:other meleic acid
               ( A ) DESCRIPTION:synthetic DNA fragment
      ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:59:
AAAAGGCCTC GAG
(2) INFORMATION FOR SEQ ID NO:60:
       ( i ) SEQUENCE CHARACTERISTICS:
              ( A ) LENGTH:14 amino acids
```

- ( B ) TYPE: amiso acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protria
- (x i ) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
-continued
     Arg Glu
                             Glu Pro Trp Gly Ala Xaa Xaa Leu Glu
                                                      10
( 2 ) INFORMATION FOR SEQ ID NO:61:
        ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:42 bases
               ( B ) TYPE: socleic acid
               ( C ) STRANDEDNESS: single
               ( D ) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE:other ancleic acid
               ( A ) DESCRIPTION: synthetic DNA fragment
      ( a i ) SEQUENCE DESCRIPTION: SEQ ID NO:61:
AMAAGGGAAG CGGCCGAGCC ATGGGGCGCC TAATAGCTCG AG
                                                                                    4 2
(2) INFORMATION FOR SEQ ID NO:62:
       ( i ) SEQUENCE CHARACTERISTICS:
               ( A ) LENGTH:66 amino acids
               (B) TYPE: amino acid
               (C) STRANDEDNESS: single
               ( D ) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE: protein
      ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:62:
Lys Arg Gio Ala Ala Glu Met Bis Ser Phe Cys Ala Phe Lys Ala Asp
      Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glo Gly Asn Gln
           Pho Giu Ser Leu Giu Giu Cys Lys Lys Met Cys Thr Arg Asp
Gly Ala
 65
(2) INFORMATION FOR SEQ ID NO:63:
       ( i ) SEQUENCE CHARACTERISTICS:
              ( A ) LENGTH:210 bases
              ( B ) TYPE: moleic acid
              ( C ) STRANDEDNESS: single
              ( D ) TOPOLOGY: linear
      ( i i ) MOLECULE TYPE other nucleic seid
              ( A ) DESCRIPTION:synthetic DNA fragment
      (x i ) SEQUENCE DESCRIPTION: SEQ ID NO:63:
AAAAGGGAAG CGGCCGAGAT GCATTCCTTC TGCGCTTTCA AAGCTGATGA
COGICCOTGI AMAGCIATCA TOMMACGITI CITCITCAMO ATTITCACGO
                                                                                  100
GTCAGTGCGA GGAATTCATT TACGGTGGTT GTGAAGGTAA CCAGAACCGG
                                                                                  150
TTCGAATCTC TAGAGGAATG TAAGAAGATG TGCACTCGTG ACGGCGCCTA
                                                                                  200
ATAGCTCGAG
                                                                                 210
(2) INFORMATION FOR SEQ ID NO:64:
```

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear

```
( i i ) MOLECULE TYPE: protein
```

(  $\mathbf{x} \cdot \mathbf{i}$  ) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Mer His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Ala Ala

1 5 10 15

Asp His Glo Arg Phe Phe Phe Asp Jie Phe Thr Arg Glo Cys Ch

20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Phe Glu Ser Leo 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp 50 55

#### (2) INFORMATION FOR SBQ ID NO:65:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - ( B ) TYPE: amino acid
  - (C) STRANDEDNESS: single (D) TOPOLOGY: finear
- ( i i ) MOLECULE TYPE: protein
- (  $\mathbf{x} \cdot \mathbf{i} \cdot$  ) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Ala Gly
1 5 10 15

Ala His Pro Arg Trp Phe Phe Asa lle Phe Thr Arg Gla Cys Gla Gla 20 25 30

Pho Sor Tyr Gly Gly Cys Gly Gly Asa Gla Asa Arg Pho Glu Ser Lou 35 40 45

Glu Glu Cys Lys Lys Met Cys Thr Arg As;

#### (2) INFORMATION FOR SEQ ID NO:66:

- ( i ) SEQUENCE CHARACTERISTICS:
  - ( A ) LENGTH:58 amino acids
  - (B) TYPE: amino acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE: protein
- ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Ser Ala 1 10 15

Ala His Pro Arg Trp Phe Phe Asa Ile Phe Thr Arg Gla Cys Gla Gla 20 25 30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Gln Ser Let 35

Giu Giu Cys Lys Lys Met Cys Thr Arg Asp 50 55

#### (2) INFORMATION FOR SBQ ID NO:67:

- ( i ) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH:97 bases
  - ( B ) TYPE: nucleic acid
  - ( C ) STRANDEDNESS: single
  - ( D ) TOPOLOGY: linear
- ( i i ) MOLECULE TYPE:other nucleic acid
  - ( A ) DESCRIPTION:synthetic DNA fragment
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO.67:

#### 71 72 -continued CCICCTAIGC AITCCTTCIG CGCCTTCAAG GCTRASRNIG GINNTIGIAR 5 0 AGSTRNICNS CNSCGTTKST TCTTCAACAT CTTCACGCGT TCCCTCC 97 ( 2 ) INFORMATION FOR SEQ ID NO:68: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH:23 bases ( B ) TYPE: nucleic acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( i i ) MOLECULE TYPE:other moleic acid ( A ) DESCRIPTION:synthetic DNA fragment (x i) SEQUENCE DESCRIPTION: SEQ ID NO:68: GGAGGGAACG CGTGAAGATG TTG 23 (2) INFORMATION FOR SEQ ID NO:69: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENOTH:28 amino acids (B) TYPE: amiso acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( i i ) MOLECULE TYPE: protein ( \* i ) SEQUENCE DESCRIPTION; SEQ ID NO:69: Phe Cys Ala Phe Lys Ala Xaa Xaa Gly Xaa Cys Xaa Xaa Xaa Xaa Xaa Arg Xaa Phe Phe Asa Ile Phe Thr Arg ( 2 ) INFORMATION FOR SEQ ID NO:70: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH:4 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: Incar ( i i ) MOLECULE TYPE: peptide ( \* i ) SEQUENCE DESCRIPTION: SEQ ID NO:70: Glu Ala Ala Glu

We cl	aim:
-------	------

1. A kallikrein inhibiting protein which comprises a non-naturally naturally occurring Kunitz domain, wherein, at each of the residues of said domain corresponding to the below identified residues of BPTL one of the following

allowed amino acids is found:		14 55		Cys, and, if residue 38 is not Cys, any conservative or semi- conservative substitution for Cys		
BPTT			15	Arg, Lys, Ala, Ser, Gly, Met,		
residue #	Allowed Amino Acid		16	Asu, Gin Ala, Gly, Ser, Asp, Asu		
10	Asp, Glu, Ala, Gly, Ser, Thr		17	Ala, Asn, Ser, Ile, Gly, Val,		
11	Asp, Gly, Ser, Val, Glu, Len,			Gln, Thr		
	Met, Asn, Ile, Ala, Thr	60	18	His, Leu, Gln, Ala		
12	Gly, and, if residue 14 or 38		19	Pro, Ghn, Leu, Asn, He		
	is not Cys, any conservative or		20	Arg, Leu, Ala, Ser, Lys, Gln,		
	semi-conservative substitution			Val		
	for a "normal" conformation Gly		21	Trp, Phe, Tyr, His, He		
13	as defined in Table 9		31	Glu, Asp, Gln, Asn, Ser, Ala,		
	Arg, His, Pro, Asn, Ser, Thr,	65		Val, Leu, Ile, Thr		
	Ala, Gly, Lys, Gln		32	Glu, Gln, Asp, Asn, Pro, Thr,		

**BPTT** 

residue #

Allowed Amino Acid

-continued

BPT1 residue #	Allowed Amino Acid	<del>_</del>
	Leu, Ser, Ala, Gly, Val	
33	Phe, Tyr	
34	Ser, Thr. Ile, Val, Ala, Asn., Gly, Leu	
35	Tyr, Trp, Phe	
36	Gly, Ser, Ala	
37	Gly, and, if residue 14 or 38 is not Cys, any conservative or semi-conservative substitution for a "normal" conformation Gly as defined in Table 9	10
38	Cys, and, if residue 14 is not Cys, any conservative or semi- conservative substitution for Cys	15
39	Gly, Ghu, Ala, Ser, Asp.	

- 2. A method of treating a disorder attributable to excessive kallikrein activity which comprises administering, to a human or animal subject who would benefit therefrom, a kallikrein-inhibitory amount of the protein of claim 1.
- 3. A method of assaying for the presence of kallikrein in a sample which method comprises: providing the protein of claim 1 in labeled or insolubilized form. contacting said protein with said sample, and determining whether a complex of said protein and kallikrein in the sample is formed.
- 4. A method of purifying kallikrein from a mixture which comprises: providing the protein of claim 1 in insolubilized 30 form, and contacting the mixture with said insolubilized protein so that kallikrein in the mixture is bound.
- 5. A kallikrein inhibiting protein which comprises a non-naturally occurring Kunitz domain, wherein, at each of the residues corresponding to the below identified residues, 35 one of the following allowed amino acids is found:

BPTI residue #	Allowed Amino Acid	_
10	Asp, Glu, Ala, Gly, Ser, Thr	
11	Asp, Gly, Ser, Val, Glu, Leu, Met	
12	Gly, and, if residue 14 or 38	
	is not Cys, or any conservative	
	semi-conservative substitution	
	for a "normal" conformation Gly	
	as defined in Table 9	
13	Arg, His, Pro, Asn, Ser	
14	Cys, and, if residue 38 is not	
	Cys, any conservative or semi-	
	conservative substitution for	:
15	Cys	
16	Arg, Lys	
17	Ala, Gly Ala, Asn, Ser, Ile	
18	His, Leu, Gh	
19	Pro, Gin, Leu	
20	Arg, Leu, Ala, Ser, Lys, Gin,	5
	Val	
21	Trp, Phe	
31	Ghu	
32	Ghu, Ghn	
33	Phe	
34	Ser, Thr. He	6
35	Tyr	
36	Gly, Ser, Ala	
37	Gly, and, if residue 14 or 38	
	not Cys, any conservative or	
	semi-conservative substitution	
	for a "normal" conformation Gly	6
	as defined in Table 9	

	BPTI residue #	Allowed Amino Acid	
5	38	Cys, and, if residue corresponding to position 14 is not Cys, any conservative or	
	39	semi-conservative substitution Gly, Glu, Ala.	

6. The protein of claim 2 wherein, the Kunitz domain is further characterized as follows:

 BPII Residue No.	Allowed Residue
10	Asp, Glu
11	Asp, Gly, Ser, Val
12	Gly
14	Cys
20	
36	Arg Gly
37	Glý
38	Cys.

- 7. A method of preventing or treating a disorder attributable to excessive kallikrein activity which comprises administering to a human or animal subject who would benefit therefrom, a kallikrein-inhibitory amount of the protein of claim 5.
- 8. A method of assaying for the presence of kallikrein in a sample which method comprises: providing the protein of claim 5 in labeled or insolubilized form, contacting said protein with said sample and determining whether a complex of said protein and kallikrein in the sample is formed.
- 9. A method of purifying kallikrein from a mixture which comprises: providing the protein of claim 5 in insolubilized form, and contacting the mixture with said insolubilized protein so that kallikrein in the mixture is bound.
- 10. A plasma kallikrein inhibiting protein which comprises a sequence that is homologous to a reference sequence being selected from the group consisting of: KKII/3#1 (SEQ ID NO. 5), KKII/3#2 (SEQ ID NO. 6), KKII/3#3 (SEQ ID NO. 7), KKII/3#4 (SEQ ID NO. 8), KKII/3#5 (SEQ ID NO. 9), KKII/3#6 (SEQ ID NO. 10), KKII/3#7 (SEQ ID NO. 11), KKII/3#8 (SEQ ID NO. 12), KKII/3#9 (SEQ ID NO. 13), KKII/3#10 (SEQ ID NO. 15), KK2/#11 (SEQ ID NO. 22), KK2/#13 (SEQ ID NO. 19), KK2/#1 (SEQ ID NO. 23), [KK2/#2, ] KK2/#3 (SEQ ID NO. 28), KK2/#4 (SEQ ID NO. 24), KK2/#6 (SEQ ID NO. 25), KK2/#7 (SEQ ID NO. 29) KK2/#10 (SEQ ID NO. 27), KK2/#12 (SEQ ID NO. 31), and KK2con1 (SEQ ID NO.32) as defined in Table 2.

A method of treating a disorder attributable to excessive kallikrein activity which comprises administering, to a human or animal subject who would benefit therefrom, a kallikrein-inhibitory amount of the protein of claim 10.

12. A method of assaying for the presence of kallikrein in a sample which method comprises: providing the protein of claim 10 in labeled or insolubilized form, contacting said protein with said sample, and determining whether a complex of said protein and kallikrein in the sample is formed.

13. A method of purifying kallikrein from a mixture which comprises: providing the protein of claim 10 in insolubilized form, and contacting the mixture with said insolubilized protein so that kallikrein in the mixture is bound.

. . .

PATENT NO. : 5,795,865

Page 1 of 2

DATED

: August 18, 1998

INVENTOR(S): Markland et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item [75] Inventors: "Willaim" should read --William--.

Column 1, line 52, after "Genebank entry" insert -- P03952--.

Column 2, line 64, after "from a" delete -.-- (period).

Column 3, line 14, "the" should read -- they--.

Column 4, line 55, "purposed" should read --purposes--.

Column 5, line 56, "S 1" should read --S1--.

Column 6, line 20, delete "]".

Column 7, line 21, "screeneing" should read --screening--; line 24 "K" should read --K;--.

Column 8, line 24, "D 16G" should read --D16G--; line 35, "S 17A" should read --S17A--; line 35 "T18H, E19P" should read -T18H, E19P-. line 41, "S 15R" should read --S15R--; line 60, "A3 1E" should read --A31E--.

Column 9, line 3, "RI18H" should read -- I18H--; line 7, "RI18H" should read -- I18H--; line 8, "MODIFICARION" should read -MODIFICATION-; line 29, "R,15" should read --R15--; line 53, after "sequences" insert -- -- {period}.

PATENT NO. : 5,795,865

Page 2 of 2

DATED

<sup>:</sup> August 18, 1998

INVENTOR(S): Marland et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11, line 23, after "small and" delete "the".

Column 14, line 56, "optimiize" should read --optimize--.

Column 15, line 31, "(F)" should read --(F)--.

Column 71, line 51, after "non-naturally" delete "naturally".

Column 73, line 44, delete "or" before "any conservative", and insert --or-- after "conservative".

Column 74, line 8, after "substitution" insert -- for Cys--; line 25, delete "preventing or"; line 33, after "said sample" insert -,- {comma}; line 48, delete "[KK2/#2, ]".

Signed and Sealed this

Ninth Day of March, 1999

Attest:

Q. TODD DICKINSON

Attesting Officer

Acting Commissioner of Patents and Trademarks

PATENT NO. : 5,795,865

Page 1 of 2

DATED

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Column 2, line 64, after "from a" delete --.- {period}.

Column 3, line 14, "the" should read --they-.

Column 4, line 55, "purposed" should read --purposes--.

Column 5, line 56, "S 1" should read --S1--.

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PATENT NO. : 5,795,865

Page 2 of 2

DATED

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INVENTOR(S): Marland et al.

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Signed and Sealed this

Ninth Day of March, 1999

Attest:

Q. TODD DICKINSON

Attesting Officer

Acting Commissioner of Patents and Trademarks

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

Attachment F

Terminal Disclaimer

## ATTACHMENT F

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ART UNIT:

**EXAMINER:** 

Degen

Application of:

Markland and Ladner

Serial No.:

08/676,125

Filed:

September 25, 1996

Entitled:

Kallikrein-Inhibiting "Kunitz

Domain" Proteins and Analogues

Thereof

Attorney Ref. DYX-006.2P US/Markland-1B

Assistant Commissioner for Patents Washington, D.C. 20231

# TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A COPENDING PATENT APPLICATION

The undersigned, being an attorney of record in the above-referenced patent application who is empowered to sign on behalf of Dyax Corp., avers that Dyax Corp., a Deleware corporation having a place of business at One Kendall Square, Building 600, Cambridge, Massachusetts 02139, certifies that it is the assignee of the entire right, title, and interest in the above-referenced patent application by virtue of the assignment from the inventors identified above as indicated by the Assignment Records of the U.S. Patent and Trademark Office at Reel No. 8151, Frame No. 0915.

Dyax Corp. hereby disclaims the terminal part of the statutory term of any patent granted on the above-identified patent application, which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. 154-156 and 173 as shortened by any terminal disclaimer filed prior to the grant of any patent granted on the copending and commonly assigned U.S. application Ser. No. 08/208,264, filed March 10, 1994. Dyax Corp. hereby agrees that any patent so granted on the above-identified application shall be enforceable only for and during such period that it and any patent granted on said copending application are commonly owned, this agreement to run with any patent granted on the above-identified application and to be binding upon the grantee, its successor or assigns.

In making the above disclaimer, Dyax Corp. does not disclaim the terminal part of any patent granted on the above-identified application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154-156 and 173 of any patent granted on the copending application, as shortened by any terminal disclaimer filed prior to the patent grant, in the event that any such granted patent: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The Commissioner is hereby authorized to charge the terminal disclaimer fee under 37 CFR 1.20(d) to Deposit Account No. 50-0268.

Respectfully submitted,

Leon R. Yankwich, Reg. No. 30,237

Attorney for Applicants Yankwich & Associates 130 Bishop Allen Drive

Cambridge, MA 02139 Telephone: (617) 491-4343

Facsimile: (617) 491-8801

In re U.S. Patent No.: 5,795,865

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

Attorney Docket No.: D2033-7060US/10280-096US1

ANALOGUES THEREOF

Attachment G

Certificate of Correction

# ATTACHMENT G



UNITED STATES PARTMENT OF COMMERCE Patent and Trades Office ASSISTANT SECRETARY OF COMMERCE AND COMMISSIONER OF PATENTS AND TRADEMARKS Washington, D.C. 20231

the state of the s		
Leon R. Yankwich	<del></del>	******
Yankwich & Associates		
130 Bishop Allen Drive		
Cambridge, MA 02139		
0 / == 02137	-	•

MAILING DATE JAN	V 1 2 1999
PATENT NO. 5,795,865	PATENT DATE August 18, 1998
PATENTEE Markliand & Ladner	
ATTORNEY DOCKET NO	•

		DOCKEI NO.	
٠.	NOTIFICATION REGARDING	REQUEST FOR CERTIFICATI	? OR COPPENTION
The Cercon	rifficate of Correction requested in the patent identified above has being as requested. The Certificate, so modified, will be issued on	an approving with the exception indica	ted below. The remaining errors will be
	A. THE CHANGES BELOW CANNOT BE INCLUDED IN	THE CERTIFICATE SINCE THE REAL	IPST WAS DIE EIN STORM
	tine is printed in accordance with the	record.	
	(a) The change referred to was initialed and dated by ap	plicant before execution of the application	papers.
	In column , line , the error resulted from a df the amendment was omitted.	pplicant's failure to comply with Rule 121	(a), in that the precise point of entry
	3. In column tine the alleged error is due to use of brackets, instead of parentheses, to cancel subject many	o applicant's failure to comply with Rule 1 er and for the use of interlineations to incli-	21(b), wherein provision is made for
	4. Omission of the priority data from the patent resulted from ap		
• • .	(a) The priority data was omitted from the eath, or declarate	ion	119, m uzc
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	(c) The conflict copy of the foreign application was not file 5. Since, the inventor name(s) leave white the state of the	d: 36 = 1	Miller Andrewski († 1865) 18 maai - Andrewski († 1865)
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	Any petition should be directed to the attention of the Assistant C By Mail: Commissioner of Patents and Trad Box DAC	ommissioner for Patents, using the followi	ng mailing address or PAX member. (703) 308-6916
	Washington, D.C. 20231		Attn: Office of Petitions
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or, Confinences of Co	hrrection Branch  This decision is rendered p	ursuant to authority deleg he Commissioner of Pater	ated by the Solicitor under	r authority delegate	d to him	

PATENT NO. : 5,795,865

DATED : August 18, 1998

Page 1 of 2

INVENTOR(S): Harkland et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby

On the title page, item [75] Inventors: "Willaim" should read

Column 1, line 52, after "Genebank entry." insert -- P03952--.

Column 2, line 64, after "from a" delete -.- {period}.

Column 3, line 14, "the" should read they.

Column 4, line 55, "purposed" should read -purposes-.

Column 5, line 56, "S 1" should read -S1-.

Column 6, line 20, delete "]".

Column 7, line 21, "screeneing" should read -screening-; line 24 "K" should read -K;-.

Column 8, line 24, "D 16G" should read -D16G-; line 35, "S 17A" should read -S17A-; line 35 "T18H, E19P" should read —T18H, E19P—.

line 41, "S 15R" should read -S15R-; line 60, "A3 1E" should read -A31E-. Column 9, line 3, "RI18H" should read -- I18H-; line 7, "RI18H" should read -- I18H-;

line 8, "MODIFICARION" should read —MODIFICATION—;

line 29, "R,15" should read -R15-; line 53, after "sequences" insert --- (period).

PATENT NO. : 5,795,865

DATED

August 18, 1998

Attesting Officer

INVENTOR(S): Marland et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11, line 23, after "small and" delete "the".

Column 14, line 56, "optimize" should read --optimize-.

Column 15, line 31, "(F)" should read -(F)-.

Column 71, line 51, after "non-naturally" delete "naturally".

Column 73, line 44, delete "or" before "any conservative", and insert -or - after "conservative".

Column 74, line 8, after "substitution" insert --for Cys-; line 25, delete "preventing or"; line 33, after "said sample" insert --, -- {comma}; line 48, delete "[KK2#2, ]".



Signed and Sealed this

Page 2 of 2

Ninth Day of March, 1999

Q. TODD DICKINSON

Acting Commissioner of Patents and Trademarks

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

Attachment H

Maintenance Fee Statement

### ATTACHMENT H



# UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 37462

ISTMT

DATE PRINTED 12/08/2009

LANDO & ANASTASI, LLP ONE MAIN STREET, SUITE 1100 CAMBRIDGE MA 02142

# MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL, FILING	PAYMENT	SMALL	ATTY DKT
5,795,865	\$880.00	\$0.00	01/24/02	08/676,125	08/18/98	DATE 09/25/96	YEAR 04	NO	NUMBER D2033-7060US

TOL-439 (Rev. 05/2006)



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Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER FEE AMT 5,795,865 \$2,300.00	SUR CHARGE \$0.00	PYMT DATE 02/21/06	U.S. APPLICATION NUMBER 08/676,125	PATENT ISSUE DATE 08/18/98	APPL. FILING DATE 09/25/96	PAYMENT YEAR 08	SMALL ENTITY? NO	ATTY DKT NUMBER D2033-7060US	: : : : : : : : : : : : : : : : : : :
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In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

#### Attachment I

Alignment of the Kunitz domain of ecallantide and bovine trypsin protease inhibitor

### **ATTACHMENT I**

 $\label{eq:mass} \begin{array}{llll} \texttt{MHSFCAFKADDGPCRAAHPRWFFNIFTRQCEEFIYGGCEGNQNRFESLEECKKMCTRD} \\ & & \texttt{GPC+A} & \texttt{R+} + \texttt{N} & \texttt{C+} & \texttt{F+YGGC} & + \texttt{N} & + \texttt{S} & \texttt{E+C} & + & \texttt{C} \\ & & \texttt{RPDECLEPPYTGPCKARIIRYEYNAKAGLCQTFVYGGCRAKRNNEKSAEDCMRTCGGA} \end{array}$ 

Alignment of the amino acid sequence of ecallantide (first line) and the amino acid sequence of bovine protease trypsin inhibitor (BPTI) (third line). The second line shows positions within the amino acid sequences that have identical amino acid residues (indicated by capital letters) and amino acid residues that are conservative amino acid substitutions between the two sequences (indicated by "+")

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

Attachment J1

Letter from FDA acknowledging receipt of the first IND

# ATTACHMENT J [

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration 1401 Rockville Pike Rockville MD 20852-1448

JAN 1 8 2002

Our Reference: BB-IND 10232

**Dyax Corporation** Attention: Lynn G. Baird, Ph.D.

Senior Vice President, Preclinical and Regulatory Affairs

300 Technology Square Cambridge, MA 02139

Dear Dr. Baird:

The Center for Biologics Evaluation and Research has received your Investigational New Drug Application (IND). The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 10232

**SPONSOR: Dyax Corporation** 

PRODUCT NAME: Plasma Kallikrein Inhibitor (recombinant, Pichia pastoris, Avecia

Biotechnology)

DATE OF SUBMISSION: January 10, 2002

DATE OF RECEIPT: January 11, 2002

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an original and two copies of every submission to this file. Please include three originals of all illustrations which do not

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-4358. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research Attn: Office of Therapeutics Research and Review HFM-99, Room 200N 1401 Rockville Pike Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Karen D. Winestock

Regulatory Project Manager

Division of Application Review and Policy

Office of Therapeutics

Research and Review

Center for Biologics

**Evaluation and Research** 

Enclosures (3): 21 CFR Part 312

21 CFR 50.20, 50.25

Information sheet on 21 CFR 25.24

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

Attachment J2

Contact Report for DYAX-FDA Teleconference of February 8, 2002

## ATTACHMENT J2

## **RECORD OF CONTACT**

**CONTACT:** Scott Proestel

DATE OF CONTACT: 08 February 2002 DATE OF REPORT: 08 February 2002

DYAX: Lynn G. Baird, Tony Williams,

IND#: 10232

**Jeff Peart** 

cc: Henry Blair, Shil Hirani, Tony Williams, Jeff Peart

**SUBJECT:** Stopping Rule

We called Scott Proestel on 08 February 2002. He acknowledged that he had received our fax. He said that our proposed stopping rule looked satisfactory to him and he was scheduled to review it with his supervisor in the next half hour or so. He would get back to us after they had conferred.

In the fax, we indicated that we would like to clarify the outcome of yesterday's discussion on replacement of patients. Scott said that all patients who are treated on study should be followed for 28 days for safety. Patients who do not receive study medication can be replaced; those who have receive study medication should not be replaced. Tony asked if we could replace a patient who was treated but from whom no PK samples were collected. Scott did not think such patients should be replaced, although he offered to discuss the matter with his supervisor if we wanted. Lynn suggested that rather than trying to define appropriate exceptions in the protocol, we consider calling Scott to discuss any patient for whom we thought replacement was justified. Tony agreed to replace only non-dosed patients and to call if he felt there were justifiable exceptions.

Scott said that he would call in a half an hour or so about the stopping rule after talking with Ellis Unger. He called and said everything looked satisfactory with the exception of one number. The proposed SAE trigger of greater than 3 of 4 was too high. They recommended setting this trigger at 2 of 4. I agreed that we would make the change. Scott said that we could proceed with our Phase I/II clinical trial. He asked that we submit the amended protocol as an IND amendment. I assured him that we would.

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

## Attachment K

Letter from FDA acknowledging receipt of the second IND

## ATTACHMENT K



## DEPARTMENT OF HEALTH & HUMAN SERVICES

MAY 1 0 2002

Food and Drug Administration 1401 Rockville Pike Rockville MD 20852-1448

Our Reference: BB-IND 10426

**Dyax Corporation** Attention: Lynn G. Baird, Ph.D. Senior Vice President, Development 300 Technology Square Cambridge, MA 02139

Dear Dr. Baird:

The Center for Biologics Evaluation and Research has received your Investigational New Drug Application (IND). The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 10426

SPONSOR: Dyax Corporation

PRODUCT NAME: Kallikrein Plasma Inhibitor (recombinant, Pichia pastoris, Avecia

Biotechnology)

DATE OF SUBMISSION: April 30, 2002

DATE OF RECEIPT: May 1, 2002

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an original and two copies of every submission to this file. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent

regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-4358 Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research Attn: Office of Therapeutics Research and Review HFM-99, Room 200N 1401 Rockville Pike Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Karen Winestock

Regulatory Project Manager

Division of Application Review and Policy

Office of Therapeutics

Research and Review

Center for Biologics

**Evaluation and Research** 

Enclosures (3): 21 CFR Part 312

21 CFR 50.20, 50.25

Information sheet on 21 CFR 25.24

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

## Attachment L

Letter from Dyax to the Center for Biologic Evaluation and Research dated May 31, 2002 which summarized the May 30, 2002 telephone conference

ATTACHMENT L

DEPARTMENT OF PUBLI	Form Approved: OMB No. 0910-0014. Expiration Date: September 30, 2002 See OMB Statement on Reverse.	
NOTESTIGATIONAL N	DOTE ADMINISTRATION IEW DRUG APPLICATION (II ERAL REGULATIONS (CFR) PART 31	
1. NAME OF SPONSOR		2. DATE OF SUBMISSION
Dyax Corp.		31 May 2002
3. ADDRESS (Number, Street, City, State as	nd Zip Code)	4. TELEPHONE NUMBER (Include Area Code)
300 Technology Square Cambridge, MA 02139		617-250-5705
5. NAME(S) OF DRUG (Include all available		6. IND NUMBER (If previously assigned) BB-IND # 10426
DX-88 (Recombinant Human Plas 7. INDICATION(S) (Covered by this submiss		
Hereditary Angioedema	ion,	
8. PHASE(S) OF CLINICAL INVESTIGATION	TOBE CONDUCTED:	·
		SE 2 PHASE 3 OTHER
9. LIST NUMBERS OF ALL INVESTIGA	ATIONAL NEW DRUG APPLICATIONS (21	(Specify)  CFR Part 312), NEW DRUG OR ANTIBIOTIC APPLICATION
(21 CFR Part 314), DRUG MASTER TO IN THIS APPLICATION.	FILES (21 CFR Part 314.420), AND PROI	DUCT LICENSE APPLICATIONS (21 CFR Part 601) REFER
BB-IND # 10232		·
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	·	
10. IND submission should be ( "Serial number: 000." The n	consecutively numbered. The initi ext submission (e.g., amendment,	ial IND should be numbered
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indinbered consecutively in the	order in which they are submitted.	$\frac{0}{0}$
11. THIS SUBMISSION CONTAINS THE FO	LLOWING: <i>(Check all that apply)</i> IAL NEW DRUG APPLICATION (IND)	RESPONSE TO CLINICAL HOLD
PROTOCOL AMENDMENT(S):	INFORMATION AMENDMENT(S):	IND SAFETY REPORT(S):
NEW PROTOCOL	CHEMISTRY/MICROBIOLOGY	☐ INITIAL WRITTEN REPORT
CHANGE IN PROTOCOL	PHARMACOLOGY/TOXICOLOGY	FOLLOW-UP TO A WRITTEN REPORT
NEW INVESTIGATOR	CUNICAL	
RESPONSE TO FDA REQUEST FOR INF	· . —	
REQUEST FOR REINSTATEMENT OF IN INACTIVATED, TERMINATED OR DISCO	D THAT IS WITHDRAWN, OT	THER (Specify)
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	12. CONTENTS OF A	
Į	1. Form FDA 1571 [21 CFR 312.23(a)(1)]	
ļ	2. Table of Contents [21 CFR 312.23(a)(2)]	•
	3. Introductory statement [21 CFR 312.23(a)(3)]	
	4. General Investigational plan [21 CFR 312.23(a)(3)]	
I	5. Investigator's brochure [21 CFR 312.23(a)(5)]	•
I	6. Protocol(s) [21 CFR 312.23(a)(6)]	
ı	a. Study protocol(s) [21 CFR 312.23(a)(6)]	
ı	b. Investigator data [21 CFR 312.23(a)(6)(iii)(b)] or	
l	☐ c. Facilities data [21 CFR 312.23(a)(6)(iii)(b)] or co	
l	d. Institutional Review Board data [21 CFR 312.23	3(a)(6)(iii)(b)] or completed Form(s) FDA 1572
l	7. Chemistry, manufacturing, and control data [21 CFR 312.23]	
l	☐ Environmental assessment or claim for exclusion	[21 CFR 312.23(a)(7)(iv)(e)]
l	8. Pharmacology and toxicology data [21 CFR 312.23(a)(8)]	
l	9. Previous human experience [21 CFR 312.23(a)(9)]	
I	☐10. Additional information [21 CFR 312.23(a)(10)]	
l	•	
l	13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT	RESEARCH ORGANIZATION? YES X NO
	IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CON	
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	Anthony H. Williams, MA, MRCP, MB, MS	
	Sr. Vice President	
	Medical Affairs and Clinical Operations	
1	15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND SAFETY OF THE DRUG	EVALUATION OF INFORMATION RELEVANT TO THE
		ynn G. Baird, PhD
		r. Vice President
	Medical Atlan's and Chinical Operations	Development
	I agree not to begin clinical investigations until 30 days after F by FDA that the studies may begin. I also agree not to begin those studies are placed on clinical hold. I agree that an requirements set fourth in 21 CFR Part 56 will be responsible for studies in the proposed clinical investigation. I agree to condure guilatory requirements.	or continue clinical Investigations covered by the IND if Institutional Review Board (IRB) that complies with the or initial and continuing review and approval of each of the
1		7. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED
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1		7. TELEPHONE NUMBER 20. DATE
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PAGE 2 OF 2

EODM EDA 1574 (40/99)

Dyax Corp.



31 May 2002

BB-IND # 10426, Serial 002

Dr. Jay Siegel
Center for Biologics Evaluation and Review
HFM-99, Room 200N
Attention: Office of Therapeutics Research and Review
1401 Rockville Pike
Rockville, MD 20852-1448

Re: DX-88 (Recombinant Human Plasma Kallikrein Inhibitor) for Treatment of

Hereditary Angioedema

Attention: Karen Winestock

Dear Dr. Siegel:

This letter is a summary of a teleconference on 30 May 2002 with Anita O'Connor and Scott Proestel, and a follow-up of a teleconference on 29 May 2002 (reference BB-IND # 10426, Serial 001). Teleconference participants representing Dyax were Lynn Baird, SVP Development, Tony Williams, SVP Medical Affairs and Clinical Operations, and Jeff Peart, Manager Regulatory Affairs.

Dr. Proestel and Ms. O'Connor had reviewed a facsimile copy of Serial 001 and had four follow-up comments/questions:

1. Regarding the requested change from an absolute milligram dose in the proposed study to a dose based on mg/ m² body surface, what numerical doses are being considered?

Dr. Williams indicated that doses from of 5, 10, 20, 40 mg/ m² body surface would be used. He had selected these doses based on the originally proposed doses of 10, 20, 40, and 80 mg and a surface area of an average person of 1.92 m². An upper limit for surface area of 2.5 m² will be used to prevent potential overdosing.

2. It is suggested that the stopping rule associated with patient death apply to all deaths rather than "medical" deaths. Alternatively, specific events leading to

death could be identified. This should not delay study enrollment as CBER review of unrelated deaths will be rapid.

Dyax agrees to write the stopping rule to include all deaths.

3. Dr. Proestel clarified that it would be acceptable to treat up to fifty patients, not episodes, in the proposed study.

Dyax agrees. However, the protocol as written allows for treatment of 48 attacks, and will be amended to treat 48 patients.

4. Dr. Proestel asked for clarification regarding how we intended to deal with potential retreatments.

Dr. Williams stated that patients would be treated under a separate protocol rather than under the existing protocol for administrative reasons.

Dr. Proestel confirmed that the clinical trial could be initiated. However, Dyax must submit a revised clinical protocol to the IND before patient enrollment begins. Dyax agreed to submit the protocol in a subsequent amendment.

Please contact me by telephone at 617-250-5705 or telefax at 617-225-2501 if you need any additional information or require any clarification.

Sincerely,

Lynh/G. Baird, Ph.D. Senior Vice President

Development

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

### Attachment M

Communication dated June 12, 2008 from Dyax Corp. to the Center for Drug Evaluation and Research discussing conveyance of BB-IND#10232 to Cubist Pharmaceuticals

## ATTACHMENT M

	DEPARTMENT OF I FOOD AND	HEALTH AND HUMAN SERVICES DRUG ADMINISTRATION	Form Approved: ON Expiration Date: Apro See OMB Statement	il 30, 2009
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			617-250-5773	•
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	N(S) (Covered by this submission			-
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TO INTHIS	APPLICATION.	FILES (21 CFR Part 314,420), AND PRODUCT LI	ICENSE APPLICATIONS (21	CFR Part 601) REFERRED
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PAGE 1 OF 2

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☐ 3. Introductory statement [21 CFR 312.			
4. General Investigational plan [21 CFF	2 212 22/21/21] 2 212 22/21/21]		
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d. Institutional Review Bo	pard data [21 CFR 312.23(a)(6)(iii)(b)] or	S) FUA 15/2	
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Andrew Sternlicht, M.D., Ph.D.			
VP of Clinical and Medical Affairs, Dyax C			
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300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



12 June 2008

IND # 10232, Serial 100

Dwayne Rieves, MD
Therapeutic Biological Products Document Room
Center for Drug Evaluation and Research
Food and Drug Administration
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: Ecallantide IV: In Patients Undergoing Cardiopulmonary Bypass Procedures Ecallantide (DX-88 [Recombinant Human Plasma Kallikrein Inhibitor])
General Correspondence: Notice of Transfer of IND # 10232

Dear Dr. Rieves:

The purpose of this submission is to notify the FDA that sponsorship of IND 10232, DX-88 (ecallantide) for the reduction of blood loss for patients undergoing cardiopulmonary bypass surgery, is being transferred to Cubist Pharmaceuticals, Inc as of June 16, 2008. All documents associated with IND 10232 have been transferred to Cubist and all active investigators will be notified that Cubist is assuming the sponsor responsibilities for ecallantide as of June 16, 2008.

On June 16, Cubist will also assume responsibility for the ongoing clinical study DX-88/16, entitled "KALAHARI 1: Kallikrein Antagonist (DX-88 [Ecallantide]) Effect on Blood Loss Associated with Heart Surgery Requiring Institution of Bypass".

In a separate submission to IND 10232, Cubist Pharmaceuticals, Inc. will notify the FDA that they assume all of the sponsor responsibilities for IND 10232 as of June 16, 2008. Included in the Cubist submission will be a completed FDA 1571 Form which indicates the contact information for Cubist.

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or Aurelie Grienenberger by telephone at 617-250-5762 or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely

Nicole D'Auteuil

Senior Director of Regulatory Affairs

Submitted in triplicate

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

### Attachment N

Communication from Dyax Corp. to the Center for Drug Evaluation and Research dated June 13, 2008, in which BB-IND#10426 was amended

## ATTACHMENT N

	DEPARTMENT OF HE	ALTH AND HUMAN SERVICES	Form Approved: OMB No. 0910-0430.
	FOOD AND DI	RUG ADMINISTRATION	Expiration Date: April 30, 2009 See OMB Statement on Reverse.
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	300 Technology Square Cambridge, MA 02139		4. TELEPHONE NUMBER (Include Area Code)
1	5. NAME(S) OF DRUG (Include all available name		617-250-5773
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	1. Form FDA 1571 [21 CFR 312.23(a)(1)]		•	
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ı	3. Introductory statement [21 CFR 312.23(a)(3)]	•	•	
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Į	4. General Investigational plan [21 CFR 312.23(a)(3)]	•		•
I	5. Investigator's brochure [21 CFR 312.23(a)(5)]	•		
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ı	a. Study protocol(s) [21 CFR 312.23(a)(6)]			
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ı	c. Facilities data [21 CFR 312.23(a)(6)(iii)(b)]	or completed Form(s) FD	A 1572	
ı	d. Institutional Review Board data [21 CFR 3	12.23(a)(6)(iii)(b)] or comp	leted Form(s) FDA	1572
ı	7. Chemistry, manufacturing, and control data [21 CFR 312	2.23(a)(7)]	•	
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300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



13 June 2008

IND # 10426, Serial 193

Badrul Chowdhury, M.D., Ph.D.
Division of Pulmonary and Allergy Products
Center for Drug Evaluation and Research
Food and Drug Administration
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: DX-88 (ecallantide) for Treatment of Angioedema
Re-submission of documentation
Letter of Authorization to Cross-Reference IND 10426

Dear Dr. Chowdhury:

We are re-submitting information that was previously submitted to the Agency. The current submission is being made due to an IND transfer to a new sponsor. Dyax Corp. has been the Sponsor of INDs for DX-88 (ecallantide) in two indications:

- IND 10426 for attacks of hereditary angioedema (Division of Pulmonary and Allergy Products); and
- IND 10232 for the reduction of blood loss for patients undergoing cardiopulmonary bypass surgery (Division of Medical Imaging and Hematology Products)

Dyax is retaining sponsorship of IND 10426, however, transferring sponsorship of IND 10232 to Cubist Pharmaceuticals, effective June 16, 2008. Due to the transfer of IND 10232, Dyax is re-submitting all Quality and Nonclinical (NC) documentation that was previously submitted to IND 10232 and cross-referenced to IND 10426. The information in the current submission is entirely duplicative to prior submissions and is provided for the purpose of ensuring that documentation previously submitted to only IND 10232 is now directly incorporated to IND 10426.

Also attached is a letter of authorization to cross-reference IND 10426 on behalf of submissions made by Cubist Pharmaceuticals in association with IND 10232.

a de Terrette de la cerca d

As agreed with the IND project manager, Akilah Green, only 1 copy of this submission is provided because there is no new information for review. The attached table of contents lists the documentation included in the current resubmission.

Future Quality and NC submissions in support of the HAE program will be fully submitted to IND 10426.

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or Aurelie Grienenberger at 617-250-5762 or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely,

Nicole D'Auteuil

Senior Director of Regulatory Affairs

Submitted in triplicate

# Table of Contents IND 10426, Serial 193 Re-Submitted Documentation & IND Letter of Authorization

IND 10426 S-193 Volume #	IND 10232 Submission date	IND 10232 Serial #	CMC/NC	Brief description
1		Not applicable	<u> </u>	1571, Cover letter, IND letter of authorization
1-3	10 Jan 2002	000	CMC/NC	Sections 7 and 8 of original IND (Volume 1, 3 and 4-Att8 only)
4	30 Jan 2002	001	NC	Clarification to animal identification numbers
5	01 Feb 2002	002	CMC	Animal origin statement
6	06 Feb 2002	003	CMC	HPLC and SDS-PAGE data of DP lots used in toxicology and clinic
7	12 Aug 2002	005	CMC	Comparability of manufacturing scale-up from 100L to 1000L
8	13 Nov 2002	011	CMC	Response to FDA request for additional DS and DP testing
9	30 Dec 2002	013	NC	Nonclinical study report
10	07 May 2003	018	CMC	DS and DP shelf life extension
11	24 Oct 2003	022	CMC	New RP-HPLC method
12	21 Apr 2004	024	NC	Laboratory animal adverse events
13	26 Apr 2004	025	CMC	DX-88 purification process modification to reduce host cell proteins
14-17	21 May 2004	027	NC	Nonclinical study reports
18	01 Jun 2004	028	NC	Nonclinical pathology review of rat deaths
19	14 Jul 2004	031	NC	Synopsis of proposed NC study to support manufacturing change
20	21 Sept 2004	035	NC	Laboratory animal adverse event
21	08 Dec 2004	038	CMC/Antib ody	Response to FDA comments regarding DS and DP testing
22-23	14 Mar 2005	039	NC	Nonclinical study report
24	15 Apr 2005	041	NC	Rationale for rat as relevant species for reproductive toxicology
25	26 Apr 2005	042	NC	Summary of nonclinical laboratory deaths as we as animal adverse event narratives
26	7 Jun 2005	043	CMC	Response to FDA request for quality information
27	20 Jun 2005	044	CMC	Qualification of master seed bank (MSB)
28-29	30 Aug 2005	046	CMC/NC	Comparability data for 1000L and 5000L manufacturing processes, changes in drug product specifications
30	04 Oct 2005	047	CMC	DX-88 extinction coefficient and ultrafiltration step in manufacturing
31-42	12 Oct 2006	053	NC	Nonclinical study reports
43	1 Nov 2006	056	CMC	DP and DS changes to specifications and reference standard data
44	16 Nov 2006	057	CMC	Response to FDA request for information following EOP1/Pre-Phase 2 Meeting
45	27 Dec 2006	060	CMC	Description of placebo
46-49	9 Apr 2007	069	NC	Annual Report to 10232, including NC study reports
50	23 Apr 2007	070	CMC	Certificate of analysis of DS and DP lots in clinical studies

Confidential 2

IND 10426 S-193 Volume#	IND 10232 Submission date	IND 10232 Serial #	CMC/NC	Brief description
51	13 Feb 2008	089	CMC	Manufacturer comparability, reference standard qualification, pH investigations
52-55	9 April 2008	094	NC	Annual Report to 10232, including NC study reports

Confidential

2

300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



#### 13 June 2008

Badrul Chowdhury, M.D., Ph.D.
Division of Pulmonary and Allergy Products
Center for Drug Evaluation and Research
Food and Drug Administration
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: Letter of Authorization to Cross Reference IND 10426

Dear Dr. Chowdhury:

Dyax Corp. authorizes the FDA to incorporate, by reference, information in IND 10426 in consideration of INDs or other regulatory submissions filed by Cubist Pharmaceuticals. IND 10426 is current and up-to-date.

Reference:

IND 10426

Authorization on behalf of:

Cubist Pharmaceuticals, Inc.

65 Hayden Ave

Lexington, MA 02421

Product:

DX-88 (ecallantide)

Application type:

IND or other regulatory submissions for ecallantide

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or Aurelie Grienenberger at 617-250-5762 or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely.

Nicole D'Auteuil

Senior Director of Regulatory Affairs

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

### Attachment O

Letter from FDA acknowledging receipt of the final submission of the BLA

## ATTACHMENT O



## DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration Rockville, MD 20857

BLA ACKNOWLEDGEMENT

Our STN: BL 125277/0

Dyax Corporation 300 Technology Square Cambridge, MA 02139

OCT 29 2008

Attention:

Nicole D'Auteuil

Senior Director, Regulatory Affairs

Dear Ms. D'Auteuil:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following:

Name of Biological Product: KALBITOR (ecallantide) Injection

Date of Application: September 23, 2008

Date of Receipt: September 23, 2008

Our Submission Tracking Number (STN): BL 125277/0

Proposed Use: Treatment of Hereditary Angioedema

If you have not already done so, promptly submit the content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format as described at <a href="http://www.fda.gov/oc/datacouncil/spl.html">http://www.fda.gov/oc/datacouncil/spl.html</a>. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action. The content of labeling must conform to the format and content requirements of revised 21 CFR 201.56-57.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration Center for Drug Evaluation and Research 5901-B Ammendale Road Beltsville, MD 20705-1266

RECEIVED BY REGULATORY 4 Nov 2008 W) T SIGNATURE All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission.

If you have any questions, call me at (301) 796-1230.

Sincerely,

Colette Jackson

Regulatory Health Project Manager

Division of Pulmonary and Allergy Products

Office of Drug Evaluation II

Center for Drug Evaluation and Research

## DEPARTMENT OF

HEALITH & HUMAN SERVICES
Food and Drug Administration
Center for Drug Evaluation Research,
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Official Business Penalty for Private Use \$300



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In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

**ANALOGUES THEREOF** 

Attachment P

Certification of Copies of Application Papers

Attorney Docket No.: D2033-7060US/10280-096US1

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No.: 5,795,865

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

**CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. §1.10** 

The undersigned hereby certifies that this document was deposited with the U.S. Postal Service on

February 16, 2010 for express mailing in accordance with § .6(a)(2).

Laurie Butler Lawrence, Reg. No. 46,593

EB 575684948 US

Mail Stop Hatch-Waxman PTE

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

# SUPPLEMENT TO THE APPLICATION FOR EXTENSION OF PATENT TERM <u>UNDER 35 U.S.C. § 156</u>

In compliance with the duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services under 37 C.F.R. § 1.765, Applicants are submitting herewith a copy of an Application for Patent Term Extension Under 35 U.S.C. §156 filed for U.S. Patent No.: 7,276,480. The Application for Patent Term Extension for U.S. Patent No.: 7,276,480 is also for KALBITOR® which was subject to regulatory review in BLA number BL 125277/0.

Two duplicate copies are being submitted herewith for the Secretary of Health and Human Services. The duplicate copies are attached as Exhibit N of the Application for Patent Term Extension Under 35 U.S.C. §156 filed for U.S. Patent No.: 7,276,480.

In re U.S. Patent No.: 5,795,865 Attorney Docket No.: D2033-7060US/10280-096US1

Issued: August 18, 1998

Inventors: William Markland and Robert

Charles Ladner

Assignee: Dyax Corp.

Title: KALLIKREIN-INHIBITING "KUNITZ DOMAIN" PROTEINS AND

ANALOGUES THEREOF

It is Applicants' understanding that no fee is required for this supplement. However, if any fee is required and is otherwise absent, please charge any deficiency to Deposit Account No. 50/2762, referencing Attorney Docket No. D2033/7060US.

Respectfully submitted,

Laurie Butler Lawrence, Reg. No. 46,593

LANDO & ANASTASI, LLP

One Main Street

Cambridge, Massachusetts 02142

United States of America Telephone: 617-395-7000 Facsimile: 617-395-7070

Date: 2/16/12

Attachments:

Application for Extension of Patent Term Under 35 U.S.C. §156 for U.S. Patent No.:

7,276,480 and two duplicate copies attached as Exhibit N

Attorney Docket No.: D2033-7058 S/10280-094003

### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No.: 7,276,480 B1

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

### **CERTIFICATE OF EXPRESS MAILING UNDER 37 C.F.R. §1.10**

The undersigned hereby certifies that this document was deposited with the U.S. Postal Service on

January 11, 2010 for express mailing in accordance with §1,6(a)(2).

Laurie Butler Lawrence, Reg. No. 46,593

EB 575484344 US

Mail Stop Patent Ext.

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

## **APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156**

Applicant, Dyax Corp., represents that it is the Assignee of the entire interest in and to United States Patent No.: 7,276,480 B1 granted to Dyax Corp. on the 2<sup>nd</sup> day of October, 2007, for 'PREVENTION AND REDUCTION OF BLOOD LOSS' by virtue of an assignment from Robert C. Ladner and Arthur C. Ley to Dyax Corp., recorded in the U.S. Patent and Trademark Office at Reel 017499, Frame 0493, on April 18, 2006.

By the Power of Attorney enclosed herein (Attachment A), Applicant has appointed several individual attorneys, including Laurie Butler Lawrence, as attorneys for Dyax Corp. with regard to this application for extension of the term of U.S. Patent No.: 7,276,480 B1 and to transact all business in the U.S. Patent and Trademark Office in connection therewith.

Dyax Corp. is the holder of the regulatory approval granted with respect to the regulatory review period relied on herein.

In re U.S. Patent No.: 7,276,480 B1 Attorney Docket No.: D2033-705812 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

## Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for extension of the patent term under 35 U.S.C. § 156 by providing the following information required by the rules promulgated by the U.S. Patent and Trademark Office (37 C.F.R. § 1.740). For the convenience of the Patent and Trademark Office, the information contained in this application will be presented herein in a format which follows the order of the requirements of Section 1.740 of Title 37 of the Code of Federal Regulations.

## (1) Identification of the Approved Product [1.740(a)(1)]

The approved product is KALBITOR® (ecallantide). Ecallantide is a recombinant 60 amino acid plasma kallikrein inhibiting protein produced in *Pichia pastoris* cells. KALBITOR® is supplied as a sterile, clear, colorless liquid which is free of preservatives for subcutaneous administration. The approved product is described in more detail in the package insert, enclosed herein as Attachment B. The amino acid sequence of ecallantide (see SEQ ID NO: 2 of U.S. Patent No.: 7,276,480 B1, provided as Attachment C) is as follows:

# (2) Federal Statute Governing Regulatory Approval of the Approved Product [1.740(a)(2)]

In re U.S. Patent No.: 7,276,480 B1 Attorney Docket No.: D2033-705812 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Lev

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

The approved product, KALBITOR®, was subject to regulatory review under, § 505(i) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 355(i)) and § 351(a) of the Public Health Service Act (42 U.S.C. § 262(a)).

094003

## (3) Date of Approval for Commercial Marketing [1.740(a)(3)]

The approved product, KALBITOR®, received permission for commercial marketing or use under Section 351 (a) of the Public Health Service Act on December 1, 2009. A copy of the FDA letter issuing Biologics License No.: 1789 is attached (Attachment D).

# (4) Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [1.740(a)(4)]

The only active ingredient in KALBITOR® is ecallantide which, on information and belief, has not been approved for commercial marketing or use under the Public Health Service Act, the Virus-Serum-Toxin Act or the Federal Food, Drug and Cosmetic Act prior to the issuance of Biologics License No.: 1789 by the Food and Drug Administration on December 1, 2009. A copy of the package insert describing the approved product is attached (Attachment B).

# (5) Statement Regarding Timeliness of Submission of Patent Term Extension Request [1.740(a)(5)]

This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the permitted 60-day period pursuant to 37 C.F.R. § 1.720(f). The last day on which this application can be submitted is January 29, 2010.

# (6) Complete Identification of the Patent for Which Extension Is Being Sought [1.740(a)(6)]

In re U.S. Patent No.: 7,276,480 B1

Attorney Docket No.: D2033-705812 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

The complete identification of the patent for which a term extension is being sought is as follows:

Inventors:

Robert C. Ladner and Arthur C. Ley

Patent No.:

7,276,480 B1

Filing Date:

December 30, 2005

Issue Date:

October 2, 2007

**Expiration Date:** 

June 6, 2023

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

# (7) Copies of the Patent for Which an Extension is Being Sought [1.740(a)(7)]

A copy of U.S. Patent No.: 7,276,480 B1 is provided as Attachment C.

Attorney Docket No.: D2033-705812 US/10280-094003

# (8) Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payments, or Reexamination Certificate [1.740(a)(8)]

- (a) U.S. Patent No.: 7,276,480 B1 was subject to a terminal disclaimer over the patent to issue from U.S. Application Serial No.: 10/456,981, filed June 6, 2003 (See Attachment E);
- (b) A Certificate of Correction was entered for U.S. Patent No.: 7,276,480 B1. A copy of the Certificate of Correction, the Request for a Certificate of Correction, and the Decision Granting Petition are attached (Attachment F);
- (c) The first maintenance fee for U.S. Patent No.: 7,276,480 B1, will be due October 3, 2011. A copy of the USPTO's on-line record of patent maintenance fees for this patent is attached (Attachment G).
- (d) U.S. Patent No.: 7,276,480 B1 has not been the subject of a reexamination proceeding.

# (9) Statement Regarding Patent Claims Relative to Approved Product [1.740(a)(9)]

- (a) The following claims of U.S. Patent No.: 7,276,480 B1 claim the approved product, KALBITOR® (ecallantide): claims 1, 2, and 4.
- (b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows that the above listed claims of U.S. Patent No.: 7,276,480 B1 claim the approved product, KALBITOR® (ecallantide).
  - (1) Description of the approved product

In re U.S. Patent No.: 7,276,480 B1 Attorney Docket No.: D2033-705812 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Lev

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

The approved product is described as follows in the package insert for KALBITOR®, a copy of which is provided as Attachment B: "KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein."

The package insert for KALBITOR® also provides that: "KALBITOR is a clear and colorless, sterile, and nonpyrogenic solution. Each vial contains 10 mg of ecallantide as the active ingredient, and the following inactive ingredients: 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, and 8 mg sodium chloride in water for injection, USP."

The amino acid sequence of ecallantide (see SEQ ID NO: 2 of U.S. Patent No.: 7,276,480 B1, provided as Attachment C) is as follows:

KALBITOR® is approved for the treatment of acute attacks of hereditary angioedema (HAE) in patients. Hereditary angioedema is described in the package insert for KALBITOR® as follows:

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on chromosome 11a and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in both the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kiningen to bradykinin by protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

the classical complement cascade is therefore not present. During attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Brandykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

(2) Description of claims 1, 2, and 4 and comparison to KALBITOR®

Attorney Docket No.: D2033-705812 US/10280-094003

The following description demonstrates the manner in which at least one claim of U.S. Patent No.: 7,276,480 B1 reads on the approved product.

- (c) Claim 1 of U.S. Patent No.: 7,276,480 B1 reads on the approved product. Claim 1 is as follows:
  - 1. An isolated polypeptide comprising the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein.

Ecallantide in the approved product is an isolated polypeptide. As is described in the product insert (see Attachment B), KALBITOR® is a solution of ecallantide in 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, 8 mg sodium chloride and water for injection, USP. The sequence of ecallantide is identical to the sequence recited in claim 1 with the addition of an initial "Glu Ala" at the N terminal end of the ecallantide polypeptide. Therefore, ecallantide comprises the sequence recited in claim 1. As is described in the product insert (see Attachment B), KALBITOR® inhibits kallikrein. Thus, the approved product meets all of the limitations of the claim and the claim covers the approved product.

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

(d) Claim 2 of U.S. Patent No.: 7,276,480 B1 reads on the approved product. Claim 2 is as follows:

2. The isolated polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).

Attorney Docket No.: D2033-705812 US/10280-

As discussed above, the approved product meets all of the limitations of clam 1. The sequence of ecallantide is identical to the sequence required in claim 2. Therefore, ecallantide comprises the sequence recited in claim 2. Thus, the approved product meets all of the limitations of the claim and the claim covers the approved product.

- (e) Claim 4 of U.S. Patent No.: 7,276,480 B1 reads on the approved product. Claim 4 is as follows:
  - 4. The isolated polypeptide of claim 2, wherein the polypeptide consists of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).

As discussed above, the approved product meets all of the limitations of claims 1 and 2. The sequence of ecallantide is identical to the sequence required in claim 4. Therefore,

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

ecallantide consists of the sequence recited in claim 4. Thus, the approved product meets all of the limitations of the claim and the claim covers the approved product.

Attorney Docket No.: D2033-705812 US/10280-094003 In re U.S. Patent No.: 7,276,480 B1 Attorney Docket No.: D2033-705812 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

# (10) Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [1.740(a)(10)]

The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

#### (a) Patent Issue Date:

U.S. Patent No.: 7,276,480 B1 issued on October 2, 2007.

# (b) IND Effective Date and IND number [35 U.S.C. §156(g)(1)(B)(i); 37 C.F.R. §1.740(a)(10)(i)(A)]

A first IND was by submitted by Dyax Corp. to the FDA and received by the FDA on January 11, 2002. It was assigned number BB-IND#10232. A copy of the letter from the FDA to Dyax Corp. providing the IND number and showing the date of receipt by the FDA of the first IND is provided in Attachment H1. BB-IND#10232 was concerned with the use of ecallantide in patients undergoing cardiopulmonary bypass procedures associated with cardiothoracic surgery (CTS). In a telephone conference between Dyax Corp. and the FDA on February 8, 2002, the FDA indicated that clinical trials under BB-IND#10232 could be initiated. A copy of "Record of Contact" memorializing that telephone conference made by Dyax Corp. is provided in Attachment H2. This exemption became effective February 8, 2002.

A second IND was submitted by Dyax Corp. to the FDA and received by the FDA on May 1, 2002. It was assigned number BB-IND#10426. A copy of the letter from the FDA to Dyax Corp. providing the IND number and showing the date of receipt by the FDA of the IND is provided in Attachment I. BB-IND#10426 was concerned with the use of ecallantide to treat angioedema, in particular hereditary angioedema (HAE). BB-IND#10426 cross referenced the earlier filed BB-IND#10232 and relied on chemistry, manufacture and control (CMC) and pre-clinical studies data provided in the earlier filed

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

IND. In a telephone conference between Dyax Corp. and the FDA on May 30, 2002, the FDA indicated that clinical trials under BB-IND#10426 could be initiated. A copy of a letter from Dyax Corp. to the Center for Biologics Evaluation and Research summarizing that call is provided in Attachment J. This exemption became effective May 30, 2002.

Attorney Docket No.: D2033-705812 US/10280-094003

Both INDs were transferred to the Center for Drug Evaluation and Research in 2003 when recombinant therapeutic proteins were transferred by the FDA.

In a communication dated June 12, 2008, the earlier IND, BB-IND#10232, was conveyed to Cubist Pharmaceuticals effective as of June 16, 2008. A copy of the communication dated June 12, 2008 from Dyax Corp. to the Center for Drug Evaluation and Research is provided in Attachment K.

By a communication dated June 13, 2008, BB-IND#10426 was amended by addition of the data it relied on from the earlier filed IND. A copy of the communication from Dyax Corp. to the Center for Drug Evaluation and Research is provided in Attachment L.

Thus, as set out above, the date that an exemption under §505(i) of the Federal Food, Drug and Cosmetic Act became effective (i.e., the date that an investigational new drug application (IND) became effective for KALBITOR®) was February 8, 2002.

# (c) BLA Submission Date [35 U.S.C. §156(g)(1)(B)(i); 37 C.F.R. §1.740(a)(10)(i)(B)]

The BLA was submitted on a rolling basis. Accordingly, the initial portion of the BLA was submitted by Dyax to the FDA on December 31, 2007. The final portion was submitted on September 23, 2008, which is the date is used in the calculations provided herein. The BLA was assigned number BL 125277/0. A copy of the letter from the FDA acknowledging receipt of the BLA application is provided as Attachment M.

### (d) BLA Issue Date [35 U.S.C. §156(g)(1)(B)(ii); 37 C.F.R. §1.740(a)(10)(i)(C)]

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

The FDA approved BLA 125277/0 authorizing the marketing of KALBITOR® on December 1, 2009. KALBITOR® was approved under the Department of Health and Human Services (DHHS) U.S. License No.: 1789. A copy of the approval letter from the FDA is provided as Attachment D.

Attorney Docket No.: D2033-705812 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

# (11) Summary of Significant Events During Regulatory Review Period [1.740(a)(11)]

Attorney Docket No.: D2033-705812 US/10280-

094003

A brief description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to KALBITOR® and the dates applicable to these significant activities are set forth in a chronology of events provided below.

	Brief Description of Significant Activities During Regulatory Review Period for DX-88 (ecallantide) for 3 page associated applications: 1) BB IND 10232 for CTS. Indication (HAE IND cross-referred to this IND for CMC and Nonclinical Information); 2) BB IND 10426 for HAE Indication; 3) BLA 125277	
Date	Significant Activity	Application
10-Jan-02	BB-IND10232 submitted to FDA	BB-IND 10232
11-Jan-02	BB-IND10232 received by FDA	BB-IND 10232
8-Feb-02	BB-IND10232 in effect	BB-IND 10232
30-Apr-02	BB IND 10426 submitted to FDA	BB-IND 10426
1-May-02	BB IND 10426 received by FDA	BB-IND 10426
30-May-02	Submission: Response to 29 May clinical teleconference BB-IND 10426 in effect	BB-IND 10426
31-May-02	Submission: Response to 30 May teleconference	BB-IND 10426
21-Nov-02	FDA Orphan Designation for HAE and AAE (Designation 02-1608)	BB-IND 10426
7-Feb-03	Submission: IND Annual Report	BB-IND 10232
29-May-03	Submission: IND Annual Report	BB-IND 10426
20-Jun-03	FDA Communication regarding transfer of biologic therapeutic products from CBER to CDER.	BB-IND 10232
26-Jun-03	Submission: Reformatted Fast Track request	BB-IND 10426
5-Aug-03	Communication from FDA: Fast Track denied	BB-IND 10426
4-Mar-04	Submission: IND Annual Report	BB-IND 10232
8-Apr-04	Teleconference with FDA regarding protocol	BB-IND 10426
13-May-04	Submission: IND Annual Report	BB-IND 10426

Attorney Docket No.: D2033-705812 US/10280-094003

In re U.S. Patent No.: 7,276,480 B1

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

	Brief Description of Significant Activities During Regulatory Review Period for DX-88 (ecallantide) for 3 associated applications: (1) BB IND 10232 for CITS Indication (HAE IND cross-referred to this IND for GMC and Nonclinical Information); (2) BB IND 10426 for HAE Indication; (3) BLA 125277	
Date	Significant Activity	Application .
10-Jun-04	Call to FDA regarding Dyax press release describing topline results of EDEMA1	BB-IND 10426
6-Jul-04	Submission: Revised IB and informed consent	BB-IND 10426
29-Jul-04	Call with FDA to discuss EDEMA2 treatments per patient	BB-IND 10426
31-Jul-04	Call to FDA regarding Fast Track denial and request for re-examination	BB-IND 10426
9-Aug-04	Submission: request for EOP2 meeting	BB-IND 10426
11-Aug-04	Submission: Fast Track request resubmitted	BB-IND 10426
7-Sep-04	Submission: Request for EOP2/Pre-BLA meeting	BB-IND 10426
21-Sep-04	FDA phoned with suggestion on Fast Track designation	BB-IND 10426
24-Sep-04	Submission: Fast Track request additional information	BB-IND 10426
18-Oct-04	EOP2 meeting via teleconference	BB-IND 10426
22-Oct-04	Call with FDA to discuss topics from EOP2 Meeting	BB-IND 10426
30-Nov-04	Pivotal trial design telecon with FDA	BB-IND 10426
8-Dec-04	Submission: Response to comments at EOP2 telecon	BB-IND 10426
10-Feb-05	Teleconference with FDA to discuss design of pivotal study	BB-IND 10426
28-Feb-05	Call to FDA requesting additional feedback on pivotal trial design	BB-IND 10426
1-Mar-05	Call to FDA to discuss EOP2 meeting delay due to further discussion on integrating intravenous and subcutaneous clinical programs.	BB-IND 10426
3-Mar-05	Call from FDA to discuss plans for a meeting between Office of Orphan Drugs and ODE VI	BB-IND 10426
4-Mar-05	Call to FDA to discuss recruitment in ongoing EDEMA2 study.	BB-IND 10426
4-Mar-05	Orphan Office called to discuss meeting with ODE VI reviewers	BB-IND 10426
10-Mar-05	Dyax called FDA to discuss the primary endpoint for EDEMA3	BB-IND 10426
30-Mar-05	Submission: IND Annual Report	BB-IND 10232
29-Apr-05	Teleconference discussing endpoint for EDEMA3	BB-IND 10426
26-May-05	Submission: IND Annual Report	BB-IND 10426
14-Jul-05	EOP2 meeting	BB-IND 10426
23-Sep-05	Call with FDA to discuss IND reviews following FDA reorganization	BB-IND 10232
3-Oct-05	FDA Communication: Comments to EDEMA3 protocol (eg DX-88/14)	BB-IND 10426
19-Oct-05	Submission: Response to FDA comments for EDEMA3 study	BB-IND 10426
20-Oct-05	Submission: DMSB report	BB-IND 10426

Attorney Docket No.: D2033-705812 US/10280-In re U.S. Patent No.: 7,276,480 B1 094003

Issued: October 2, 2007

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Assignee: Dyax Corp.
Title: PREVENTION AND REDUCTION OF BLOOD LOSS

	Brief Description of Significant Activities During Regulatory Review Period for DX-88 (ecallantide) for 3 associated applications: 1) BB IND 10232 for CTS Indication (HAE IND cross-referred to this IND for CMC and Nonclinical Information); 2) BB IND 10426 for HAE Indication; 3) BLA 125277	
Date	Significant Activity	Application
27-Dec-05	Submission: Information supporting the proposed modification of the Fast Track objective	BB-IND 10426
10-Feb-06	Submission: New Fast Track request	BB-IND 10426
17-Mar-06	Submission: IND Annual Report	BB-IND 10232
30-May-06	Submission: IND Annual Report	BB-IND 10426
29-Aug-06	Type B meeting	BB-IND 10426
3-Oct-06	Meeting: Type B meeting	BB-IND 10232
18-Oct-06	Submission: Request for Fast Track Designation	BB-IND 10426
20-Nov-06	FDA Letter: Fast Track approval	BB-IND 10426
9-Jan-07	FDA sent comments to CTS clinical protocol DX88/16	BB-IND 10232
11-Jan-07	FDA Communication: Meeting minutes from 13 Dec 06 meeting	BB-IND 10426
17-Jan-07	Type A Meeting via teleconference regarding SPA for EDEMA4	BB-IND 10426
13-Feb-07	Submission: Responses to FDA comments to CTS clinical protocol DX88/16	BB-IND 10232
9-Apr-07	Submission: IND Annual Report	BB-IND 10232
29-May-07	Submission: IND Annual Report	BB-IND 10426
13-Jun-07	Submission: Type C Briefing Package for 16 July 2007 meeting to discuss Filability based on positive Phase 3 (EDEMA3) results	BB-IND 10426
12-Jul-07	FDA Communication: Draft Responses to Questions for 16 July 07 meeting regarding filing on EDEMA3 package	BB-IND 10426
1-Aug-07	Submission: preBLA Type B Meeting Request	BB-IND 10426
9-Aug-07	Submission: Proprietary name review request	BB-IND 10426
1-Oct-07	Submission: Pre-BLA Briefing Book for the October 30th, 2007 meeting	BB-IND 10426
24-Oct-07	FDA Letter: SPA Agreement	BB-IND 10426
30-Oct-07	Pre-BLA meeting	BB-IND 10426
19-Nov-07	Email to FDA requesting BLA number	BB-IND 10426
19-Nov-07	Email submitting Dyax information to FDA to obtain BLA	BB-IND 10426
20-Nov-07	FDA Letter: Assignment of BLA number	BB-IND 10426
20-Nov-07	Assignment and confirmation of BLA from FDA	BB-IND 10426
20-Dec-07	Submission: Rolling Review Request	BB-IND 10426
31-Dec-07	Submission: CMC rolling submission	BLA125277

Issued: October 2, 2007

Attorney Docket No.: D2033-705812 US/10280-

094003

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

	Brief Description of Significant Activities During Regulatory Review Period for DX-88 (ecallantide) for 3 associated applications: 1) BB IND 10232 for CTS Indication (HAE IND cross-referred to this IND for CMC and Nonclinical Information); 2) BB IND 10426 for HAE Indication; 3) BLA 125277	
Date.	Significant/Activity	Application
18-Jan-08	FDA Communication: Acceptance of submission of rolling sections of BLA	BB-IND 10426
27-Mar-08	Submission: Nonclinical rolling submission	BLA125277
9-Apr-08	Submission: IND Annual Report	BB-IND 10232
7-May-08	Submission: IND Annual Report	BB-IND 10426
12-Jun-08	Submission: Notification to FDA that BB-IND 10232 was transferred to Cubist Pharmaceuticals, effective 16June2008	BB-IND 10232
13-Jun-08	Submission: Copied to BB-IND 10426 the CMC and nonclinical submissions that had previously been submitted to BB-IND 10232. The submission ensured that from this point forward BB-IND 10426 no longer relied on BB-IND 10232 for CMC and nonclinical.	BB-IND 10426
23-Sep-08	Submission: Original BLA submission completed (starting PDUFA clock)	BLA125277
10-Oct-08	Submission: Response to Office of Compliance questions	BLA125277
24-Oct-08	Teleconference regarding Pre-Approval Inspection of drug substance facility	BLA125277
17-Nov-08	Teleconference regarding Pre-Approval Inspection of drug substance facility	BLA125277
20-Nov-08	FDA Letter: Filing of the BLA including initial review comments/questions	BLA125277
8-Jan-09	Teleconference regarding advisory committee topics	BLA125277
25-Mar-09	FDA Action Letter: Complete Response	BLA125277
23-Apr-09	Submission: IND Annual Report	BB-IND 10426
31-May-09	Submission: BLA resubmission	BLA125277
5-Jun-09	FDA Letter: Acknowledgment of BLA resubmission receipt	BLA125277
5-Aug-09	FDA fax with vial/carton comments	BLA125277
12-Aug-09	Submission: Response to vial/carton label comments	BLA125277
4-Sep-09	Communication from FDA indicating preliminary acceptability of tradename	BLA125277
7-Oct-09	Teleconference regarding proposed REMS	BLA125277
16-Oct-09	FDA Letter regarding REMS requirements	BLA125277
16-Oct-09	FDA Communication with labeling comments	BLA125277
20-Oct-09	Teleconference regarding labeling	BLA125277
26-Oct-09	Submission: Proposed REMS with revisions per FDA Communication of 16 October 2009	BLA125277

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Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

	Brief Description of Significant Activities Durings Regulatory Review Periodifor Dx-88 (ecallantide) for 3 associated applications: (1) BB IND 10232 for CTS Indication (HAE IND cross-referred to this IND for CMC and Nonclinical Information): (2) BB IND 10426 for HAE Indication; 3) BLA 125277	
Date:	Significant Activity	Application
29-Oct-09	FDA Letter: B36 BLA acknowledgement	BLA125277
19-Nov-09	FDA Communication with REMS comments	BLA125277
20-Nov-09	FDA Communication with labeling comments	BLA125277
24-Nov-09	Teleconference regarding post marketing requirements	BLA125277
1-Dec-09	FDA Action Letter: BLA Approval	BLA125277

Issued: October 2, 2007

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Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

# (12) Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. §1.740(a)(12)]

- (i) Applicant is of the opinion that U.S. Patent No.: 7,276,480 B1 is eligible for extension of the patent term under 35 U.S.C. § 156 of 178 days and should be extended until December 1, 2023. It satisfies all requirements for such extension including:
  - (a) 35 U.S.C. § 156(a) U.S. Patent No.: 7,276,480 B1 claims KALBITOR®).

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- (b) 35 U.S.C. § 156(a)(1) U.S. Patent No.: 7,276,480 B1 has not expired before submission of this application.
- (c) 35 U.S.C. § 156(a)(2) The term of U.S. Patent No.: 7,276,480 B1 has never been extended under 35 U.S.C. § 156(e)(1).
- (d) 35 U.S.C. § 156(a)(3) The application for patent term extension is submitted by the owner of record of the patent in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d) and the rules of the Patent and Trademark Office.
- (e) 35 U.S.C. § 156(a)(4) The product KALBITOR® has been subject to a regulatory review period before its commercial marketing or use.
- (f) 35 U.S.C. § 156(a)(5)(A) The commercial marketing or use of the product KALBITOR® after the regulatory review period is the first permitted commercial marketing or use under the provisions of § 351 (a) of the Public Health Service Act under which such regulatory review period occurred.
- (g) 35 U.S.C. § 156(c)(4) No other patent has been extended for the same regulatory review period for the product KALBITOR®.
- (h) This application is being submitted within 60 days of regulatory agency approval.
- (i) This application otherwise complies with all requirements of 35 U.S.C. § 156 and all applicable rules and procedures.

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(12)(ii) Applicant respectfully submits that the length of the extension of patent term for U.S. Patent No.: 7,276,480 B1 is 178 days pursuant to 35 U.S.C. § 156(c).

The length of the extension was determined pursuant to 37 C.F.R. § 1.775 as follows (the remainder of this section (12)(ii) is numbered so as to correspond to the numbering in 37 C.F.R. § 1.775):

- (c) The regulatory review period under 35 U.S.C. § 156(g)(1)(B) is a total of 2855 days, which is the sum of (1) and (2) below:
- (1) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), which is the number of days in the period beginning on the date the exemption became effective (February 8, 2002) and ending on the date an application was initially submitted (September 23, 2008), which is 2420 days; and
- (2) The period of review under 35 U.S.C. § 156(g)(1)(B)(ii), which is the number of days in the period beginning on the date the application was initially submitted (September 23, 2008) and ending on the date such application was approved (December 1, 2009), which is 435 days.
- (d) The term of the patent as extended for a human drug, antibiotic drug or human biological product is determined by:
- (1) Subtracting from the number of days determined to be in the regulatory review period, which is 2855:
- (i) The number of days in the regulatory review period which were on or before the date on which the patent issued (October 2, 2007) which is 2063 days; and
- (ii) The number of days in the period of (c)(1) and (c)(2) above during which applicant did not act with due diligence, which is zero (0) days; and

(iii) One-half the number of days determined in subparagraph (c)(1) above after that period is reduced by subparagraph (d)(1)(i) and (d)(1)(ii) which, is [[2420-2063-0]/2], or 178.5 days (wherein half days are to be ignored

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for purposes of subtraction).

Thus, the number of days determined in subparagraph (c) above (2855) is reduced by 2241 (2063+0+178) days, for a total of 614 days;

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- (2) Adding the number of days as determined in subparagraph (d)(1), (614 days), to the original term of the patent (June 6, 2023) which results in the date of February 9, 2025.
- (3) By adding fourteen (14) years to the date of issuance of the Biologics License (December 1, 2009) which results in the date of December 1, 2023;
- (4) By comparing the dates for the ends of the periods obtained pursuant to paragrapghs (d)(2) and (d)(3) and selecting the earlier, which is December 1, 2023;
- (5) (i) Since U.S. Patent No.: 7,276,480 B1 issued after September 24, 1984, by adding 5 years to the original expiration date of the patent or any earlier date set by terminal disclaimer, which results in a date of June 6, 2028; and (ii) By comparing the dates obtained pursuant to paragraphs (d)(4) and (d)(5)(i) of this section with each other and selecting the earlier date, which is December 1, 2023.

Thus, the patent is entitled to extension until December 1, 2023.

#### (13) Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Applicant acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, e.g., as that duty is defined in 37 C.F.R. § 1.765.

### (14) Applicable Fee [1.740(a)(14)]

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The prescribed fee for receiving and acting upon this application is attached as a check in the amount of \$1,120.00. The Director is authorized to charge any additional fees required by this application to Deposit Account No.: 50/2762, referencing attorney docket number D2033-705812.

#### (15)Name and Address for Correspondence [1.740(a)(15)]

All correspondence and inquiries may be directed to the undersigned, whose address, telephone number and fax number are as follows:

> Laurie Butler Lawrence Lando & Anastasi, LLP One Main Street Cambridge, MA 02142 Phone: 617-395-7000

Fax: 617-395-7070

Enclosed is a certification that the application for extension of patent term under 35 U.S.C. § 156 including its attachments and supporting papers is being submitted as one original and two (2) copies thereof (Attachment N) in compliance with 37 C.F.R. § 1.740(b).

Respectfully submitted,

Attorney Docket No.: D2033-705812 US/10280-094003

Laurie Butler Lawrence, Reg. No.: 46,593

LANDO & ANASTASI, LLP

One Main Street

Cambridge, Massachusetts 02142

United States of America Telephone: 617-395-7000 Facsimile: 617-395-7070

Attorney Docket No.: D2033-705812 US/10280-

094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

#### Attachments:

Power of Attorney (Attachment A)

Package Insert for KALBITOR® (Attachment B)

U.S. Patent No.: 7,276,480 B1, (Attachment C)

Biologics License Approval Letter including enclosures(Attachment D)

Terminal Disclaimer (Attachment E)

Certificate of Correction (Attachment F)

Maintenance Fees Status (Attachment G)

Letter from FDA acknowledging receipt of the first IND (Attachment H1)

Contact Report for DYAX-FDA Teleconference of February 8, 2002 (Attachment

H2)

Letter from FDA acknowledging receipt of the second IND (Attachment I)

Letter from Dyax to the Center for Biologic Evaluation and Research dated May

31, 2002 which summarized the May 30, 2002 telephone conference (Attachment J)

Communication dated June 12, 2008 from Dyax Corp. to the Center for Drug

Evaluation and Research discussing conveyance of BB-IND#10232 to Cubist

Pharmaceuticals (Attachment K)

Communication from Dyax Corp. to the Center for Drug Evaluation and Research dated June 13, 2008, in which BB-IND#10426 was amended (Attachment L)

Letter from FDA acknowledging receipt of the final submission of the BLA (Attachment M)

Certification of Copies of Application Papers (Attachment N)

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

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Attachment A

Power of Attorney

## ATT \CHMENT A

Docket No.: D2033-9000

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

# REVOCATION OF PRIOR POWERS OF ATTORNEY and NEW POWER OF ATTORNEY

Sir:

The undersigned, Dyax Corp., a Delaware Corporation, assignee of the entire right, title and interest for all of the patents and patent applications identified in the attached Schedule A, hereby revokes all previous powers of attorney or authorizations of agent given in the identified patents and patent applications and in any divisional, continuing, substitute, renewal, reexamination, or reissue applications thereof, and appoints all practitioners of Lowrie, Lando & Anastasi, LLP associated with Customer Number:

# 37462

as assignee's attorneys or agents with full power of substitution to take any and all action necessary with regard to the identified patents and patent applications, and with regard to any divisional, continuing, substitute, renewal or reissue applications thereof.

Please address all telephone calls to Laurie Butler Lawrence at telephone no. (617) 395-7000.

Please forward all correspondence to the correspondence address associated with Customer Number:

37462

Dyax Ørp.

Name: Ivana Magovcevic

Title: General Counsel and

Executive Vice President of Administration

# **ASSIGNEE CERTIFICATION**

Attached to this power is a Certificate Under 37 CFR 3.73(b).

/Laurie Butler Lawrence/ Laurie Butler Lawrence, Reg. No. 46,593 LOWRIE, LANDO & ANASTASI, LLP Riverfront Office Park One Main Street Cambridge, MA 02142 (617) 395-7000

# SCHEDULE A

# U.S. Patents:

-		•
U.S. PATENT NO	ISSUE DATE	ATTORNEY'S
7,244,592	07/17/2007	SOCIETINO.
7,273,610	09/25/2007	D2033-701510
6,238,860	05/29/2001	D2033-702910
6,291197	09/18/2001	D2033-703719
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6,462,172	02/03/2000	D2033-703819
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6,326,155	12/04/2001	D2033-704210
7,041,790	05/09/2006	D2033-704319
7,329,737	02/12/2008	D2033-704510
7,166,576	01/23/2007	D2033-705010
7,064,107	06/20/2006	D2033-705710
7,276,480	10/02/2007	D2033-705810
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6,333,402	12/25/2001	D2033-706030
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6,423,498	07/23/2002	D2033-706241
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6,010,880	01/04/2000	D2033-706243
6,197,526	03/09/2001	D2033-7062US
6,492,105	12/10/2002	D2033-706319
7,112,438	09/26/2006	D2033-706340
6,774,209	08/10/2004	D2033-706419
6,984,373	01/10/2006	D2033-706519
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6,989,369		D2033-706910
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7,153,829	07/19/2005	D2033-707519
7,235,530	12/26/2006	D2033-707920
·	06/26/2007	D2033-708410

# SCHEDULE A

# U.S. Patent Applications:

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U.S.		
APPLICATION NO.	PH BIO SA	ATTORNEY'S
60//337,755	. TO DATE	= <u></u>
10/313,822	12/07/2001	D2033-700200
60/337,482	12/06/2022	D2033-700210
60/336,672	12/03/2001	D2033-700300
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11/763,251	03/07/2002	D2033-701500
60/408,624	06/14/2007	D2033-701540
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. 11/796,272	04/27/2007	D2033-705811
11/929,729	10/30/2007	D2033-705813
11/860,853	09/25/2007	D2033-705814
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11/934,181	10/31/2007	D2003-705840
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10/016,329	03/01/2006	D2033-706020
08/208,265	10/26/2001	D2033-706041
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60/367,373	04/18/2002	D2033-706610
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11/932,098	10/31/2007	D2033-708425
11/932,015	10/31/2007	D2033-708426
11/932,184	10/31/2007	D2033-708427
11/931,941	10/31/2007	D2033-708428
12/020,140	01/25/2008	D2033-708520
11/199,739	08/09/2005	D2033-708531
60/754,903	12/29/2005	D2033-708600
11/646,148	12/27/2006	D2033-708610
60/755,376	12/30/2005	D2033-708700
60/805,567	06/22/2006	D2033-708701
60/870,566	12/15/2006	D2033-708702
11/648,423	12/29/2006	D2033-708710
09/988,899	11/19/2001	D2033-708820
11/862,791	09/27/2007	D2033-708830
60/198,069	04/17/2000	D2033-708900
09/837,306	04/17/2001	D2033-708910
10/000,516	10/24/2001	D2033-708930
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60/256,380	12/18/2000	D2033-709100
10/026,925	12/18/2001	D2033-709110
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60/852,263	10/17/2006	D2033-709400
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1/346,403	02/01/2006	D2033-709610
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		-2033-710000

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attorney Docket No.: D2033-7( 2 US/10280-094003

Attachment B

Package Insert for KALBITOR®

#### HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use KALBITOR® safely and effectively. See full prescribing information for KALBITOR.

KALBITOR (ecallantide) injection, for subcutaneous use Initial U.S. Approval: 2009

#### WARNING: ANAPHYLAXIS

See full prescribing information for complete boxed warning

Anaphylaxis has been reported after administration of KALBITOR®. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)].

#### INDICATIONS AND USAGE-

KALBITOR is a plasma kallikrein inhibitor indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older. (1)

#### -DOSAGE AND ADMINISTRATION-

- 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If an attack persists, an additional dose of 30 mg may be administered within a 24 hour period. (2.1)
  - KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. (2.2).

#### DOSAGE FORMS AND STRENGTHS

Single use glass vial containing 10 mg/mL of ecallantide as a solution for injection. (3)

#### -CONTRAINDICATIONS-

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR (4)

#### -WARNINGS AND PRECAUTIONS-

Hypersensitivity Reactions Including Anaphylaxis: Anaphylaxis has occurred in 3.9% of treated patients. Administer KALBITOR in a setting equipped to manage anaphylaxis and hereditary angioedema. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, monitor patients closely for hypersensitivity reactions (5).

#### -ADVERSE REACTIONS-

The most common adverse reactions occurring in ≥3% of KALBITORtreated patients and greater than placebo are headache, nausea, diarrhea, pyrexia, injection site reactions, and nasopharyngitis. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Dyax Corp. at 1-888-452-5248 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide

Revised: 12/2009

#### **FULL PRESCRIBING INFORMATION: CONTENTS\*** WARNING: ANAPHYLAXIS

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- DOSAGE AND ADMINISTRATION
  - Recommended Dosing
  - **Administration Instructions**
- DOSAGE FORMS AND STRENGTHS
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<sup>\*</sup>Sections or subsections omitted from the full prescribing information are not listed.

# **FULL PRESCRIBING INFORMATION**

## **WARNING: ANAPHYLAXIS**

Anaphylaxis has been reported after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR. [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)]

# 1 INDICATIONS AND USAGE

KALBITOR® (ecallantide) is indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older.

# 2 DOSAGE AND ADMINISTRATION

### 2.1 Recommended Dosing

The recommended dose of KALBITOR is 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If the attack persists, an additional dose of 30 mg may be administered within a 24 hour period.

### 2.2 Administration Instructions

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema.

KALBITOR should be refrigerated and protected from the light. KALBITOR is a clear, colorless liquid; visually inspect each vial for particulate matter and discoloration prior to administration. If there is particulate matter or discoloration, the vial should not be used.

Using aseptic technique, withdraw 1 mL (10 mg) of KALBITOR from the vial using a large bore needle. Change the needle on the syringe to a needle suitable for subcutaneous injection. The recommended needle size is 27 gauge. Inject KALBITOR into the skin of the abdomen, thigh, or upper arm. Repeat the procedure for each of the 3 vials comprising the KALBITOR dose. The injection site for each of the injections may be in the same or in different anatomic locations (abdomen, thigh, upper arm). There is no need for site rotation. Injection sites should be separated by at least 2 inches (5 cm) and away from the anatomical site of attack.

The same instructions apply to an additional dose administered within 24 hours. Different injection sites or the same anatomical location (as used for the first administration) may be used.

### 3 DOSAGE FORMS AND STRENGTHS

KALBITOR is a clear, colorless liquid free of preservatives. Each vial of KALBITOR contains ecallantide at a concentration of 10 mg/mL.

#### 4 CONTRAINDICATIONS

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. [see Warnings and Precautions (5.1)].

### 5 WARNINGS AND PRECAUTIONS

## 5.1 Hypersensitivity Reactions, Including Anaphylaxis

Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR. In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical studies, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruritus, rhinorrhea, sneezing, nasal congestion, throat irritation, urticaria, wheezing, and hypotension. These reactions occurred within the first hour after dosing.

Other adverse reactions indicative of hypersensitivity reactions included the following: pruritus (5.1%), rash (3.1%), and urticaria (2.0%).

Patients should be observed for an appropriate period of time after administration of KALBITOR, taking into account the time to onset of anaphylaxis seen in clinical trials. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

KALBITOR should not be administered to any patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4)].

#### 6 ADVERSE REACTIONS

Hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR [see Contraindications (4) and Warnings and Precautions (5.1)].

## 6.1 Clinical Trials Experience

Because clinical trials are conducted under varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety data described below reflect exposure to KALBITOR in 255 patients with HAE treated with either intravenous or subcutaneous KALBITOR. Of the 255 patients,

66% of patients were female and 86% were Caucasian. Patients treated with KALBITOR were between the ages of 10 and 78 years.

Overall, the most common adverse reactions in 255 patients with HAE were headache (16.1%), nausea (12.9%), fatigue (11.8%), diarrhea (10.6%), upper respiratory tract infection (8.2%), injection site reactions (7.4%), nasopharyngitis (5.9%), vomiting (5.5%), pruritus (5.1%), upper abdominal pain (5.1%), and pyrexia (4.7%). Anaphylaxis was reported in 3.9% of patients with HAE. Injection site reactions were characterized by local pruritus, erythema, pain, irritation, urticaria, and/or bruising.

The incidence of adverse reactions below is based upon 2 placebo-controlled, clinical trials (EDEMA3® and EDEMA4®) in a total of 143 unique patients with HAE. Patients were treated with KALBITOR 30 mg subcutaneous or placebo. Patients were permitted to participate sequentially in both placebo-controlled trials; safety data collected during exposure to KALBITOR was attributed to treatment with KALBITOR, and safety data collected during exposure to placebo was attributed to treatment with placebo. Table 1 shows adverse reactions occurring in ≥3% of KALBITOR-treated patients that also occurred at a higher rate than in the placebo-treated patients in the two controlled trials (EDEMA3 and EDEMA4) of the 30 mg subcutaneous dose.

Table 1: Adverse Reactions Occurring at ≥3% and Higher than Placebo in 2 Placebo Controlled Clinical Trials in Patients with HAF Treated with KAI BITOR

	KALBITOR N=100	Placebo N=81
Adverse Reactions	n (%) <sup>s</sup>	n (%) <sup>a</sup>
Headache	8 (8%)	6 (7%)
Nausea	5 (5%)	1 (1%)
Diarrhea	4 (4%)	3 (4%)
Pyrexia	4 (4%)	3 (470)
Injection site reactions	3 (3%)	1 (1%)
Nasopharyngitis	3 (3%)	1 (170)

Patients experiencing more than 1 event with the same preferred term are counted only once for that preferred term.

Some patients in EDEMA3 and EDEMA4 received a second, open-label 30 mg subcutaneous dose of KALBITOR within 24 hours following the initial dose. Adverse reactions reported by these patients who received the additional 30 mg subcutaneous dose of KALBITOR were consistent with those reported in the patients receiving a single dose.

## 6.2 Immunogenicity

In the KALBITOR HAE program, patients developed antibodies to KALBITOR. Rates of seroconversion increased with exposure to KALBITOR over time. Overall, 7.4% of patients seroconverted to anti-ecallantide antibodies. Neutralizing antibodies to ecallantide were determined *in vitro* to be present in 4.7% of patients.

Anti-ecallantide and anti-P. pastoris IgE antibodies were also detected. Patients who seroconvert may be at a higher risk of a hypersensitivity reaction. The long-term effects of antibodies to KALBITOR are not known.

The test results for the ecallantide program were determined using one of two assay formats: ELISA and bridging electrochemiluminescence (ECL). As with all therapeutic proteins, there is a potential for immunogenicity with the use of KALBITOR. The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to KALBITOR with the incidence of antibodies to other products may be misleading.

#### 7 DRUG INTERACTIONS

No formal drug interactions studies were performed. No in vitro metabolism studies were performed.

# 8 USE IN SPECIFIC POPULATIONS

## 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled trials of KALBITOR in pregnant women. KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Because animal reproductive studies are not always predictive of human response, KALBITOR should be used during pregnancy only if clearly needed.

In rats, intravenous KALBITOR at an intravenous dose approximately 13 times the maximum recommended human dose (MRHD) on a mg/kg basis caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. No development toxicity was observed in rats that received an intravenous dose approximately 8 times the MRHD on a mg/kg basis. There were no adverse effects of KALBITOR on embryofetal development in rats that received subcutaneous doses up to approximately 2.4 times the MRHD on an AUC basis, and in rabbits that received intravenous doses up to approximately 6 times the MRHD on an AUC basis.

## 8.2 Labor and Delivery

No information is available on the effects of KALBITOR during labor and delivery.

## 8.3 Nursing Mothers

It is not known whether ecallantide is excreted in human milk. Caution should be exercised when ecallantide is administered to a nursing woman.

#### 8.4 Pediatric Use

Safety and effectiveness of KALBITOR in patients below 16 years of age have not been established.

#### 8.5 Geriatric Use

Clinical trials of KALBITOR did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

#### 10 OVERDOSAGE

There have been no reports of overdose with KALBITOR. HAE patients have received single doses up to 90 mg intravenously without evidence of dose-related toxicity. No deaths occurred in monkeys that received intravenous or subcutaneous doses up to 25 mg/kg (approximately 22 times the MRHD on an AUC basis).

#### 11 DESCRIPTION

KALBITOR (ecallantide) is a human plasma kallikrein inhibitor for injection for subcutaneous use.

KALBITOR is a clear and colorless, sterile, and nonpyrogenic solution. Each vial contains 10 mg ecallantide as the active ingredient, and the following inactive ingredients: 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, and 8 mg sodium chloride in water for injection, USP. KALBITOR is preservative free, with a pH of approximately 7.0. A 30 mg dose is supplied as 3 vials each containing 1 mL of 10 mg/mL KALBITOR. Each vial contains a slight overfill. Vials are intended for single use. Ecallantide is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology.

### 12 CLINICAL PHARMACOLOGY

#### 12.1 Mechanism of Action

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on Chromosome 11q and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kininogen to bradykinin by the protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During

attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Bradykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein. KALBITOR binds to plasma kallikrein and blocks its binding site, inhibiting the conversion of HMW kininogen to bradykinin. By directly inhibiting plasma kallikrein, KALBITOR reduces the conversion of HMW kininogen to bradykinin and thereby treats symptoms of the disease during acute episodic attacks of HAE.

## 12.2 Pharmacodynamics

No exposure-response relationships for KALBITOR to components of the complement or kallikrein-kinin pathways have been established.

The effect of KALBITOR on activated partial thromboplastin time (aPTT) was measured because of potential effect on the intrinsic coagulation pathway. Prolongation of aPTT has been observed following intravenous dosing of KALBITOR at doses ≥20 mg/m². At 80 mg administered intravenously in healthy subjects, aPTT values were prolonged approximately two-fold over baseline values and returned to normal by 4 hours post-dose.

For patients taking KALBITOR, no significant QT prolongation has been seen. In a randomized, placebo-controlled trial (EDEMA4) studying the 30 mg subcutaneous dose versus placebo, 12-lead ECGs were obtained at baseline, 2 hours and 4 hours post-dose (covering the time of expected C<sub>max</sub>), and at follow-up (day 7). ECGs were evaluated for PR interval, QRS complex, and QTc interval. KALBITOR had no significant effect on the QTc interval, heart rate, or any other components of the ECG.

#### 12.3 Pharmacokinetics

Following the administration of a single 30 mg subcutaneous dose of KALBITOR to healthy subjects, a mean ( $\pm$  standard deviation) maximum plasma concentration of 586  $\pm$  106 ng/mL was observed approximately 2 to 3 hours post-dose. The mean area under the concentration-time curve was 3017  $\pm$  402 ng\*hr/mL. Following administration, plasma concentration declined with a mean elimination half-life of 2.0  $\pm$  0.5 hours. Plasma clearance was 153  $\pm$  20 mL/min and the volume of distribution was 26.4  $\pm$  7.8 L. Based on a population pharmacokinetic analysis, body weight, age, and gender were not found to affect KALBITOR exposure significantly. Ecallantide is a small protein (7054 Da) and renal elimination in the urine of treated subjects has been demonstrated.

No pharmacokinetic data are available in patients or subjects with hepatic or renal impairment.

#### 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There are no animal or human studies to assess the carcinogenic or mutagenic potential of KALBITOR (ecallantide).

KALBITOR had no effects on fertility and reproductive performance in rats at subcutaneous doses up to 25 mg/kg/day (approximately 21 times the MRHD on a mg/kg basis).

### 13.2 Animal Toxicology

Reproductive Toxicology Studies

KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Treatment of rats with an intravenous dose of 15 mg/kg/day (approximately 13 times the MRHD on a mg/kg basis) caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. However, no development toxicity was observed in rats that received an intravenous dose of 10 mg/kg/day (approximately 8 times the MRHD on a mg/kg basis). KALBITOR was not teratogenic in rats at subcutaneous doses up to 20 mg/kg/day (approximately 2.4 times the MRHD on an AUC basis) and rabbits that received intravenous doses up to 5 mg/kg/day (approximately 6 times the MRHD on an AUC basis).

#### 14 CLINICAL STUDIES

The safety and efficacy of KALBITOR was evaluated in 2 randomized, double-blind, placebo-controlled trials (EDEMA4 and EDEMA3) in 168 patients with HAE. Patients having an attack of hereditary angioedema, at any anatomic location, with at least 1 moderate or severe symptom, were treated with 30 mg subcutaneous KALBITOR or placebo. Because patients could participate in both trials, a total of 143 unique patients participated. Of the 143 patients, 94 were female, 123 were Caucasian, and the mean age was 36 years. There were 64 patients with abdominal attacks, 55 with peripheral attacks, and 24 with laryngeal attacks.

In both trials, the effects of KALBITOR were evaluated using the Mean Symptom Complex Severity (MSCS) score and the Treatment Outcome Score (TOS). These measures evaluated the severity of attack symptoms at all anatomical locations (MSCS score) and response to therapy (TOS).

MSCS score is a point-in-time measure of symptom severity. At baseline, 4 hours, and 24 hours, patients rated the severity on a categorical scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for symptoms at each affected anatomical location. Ratings were averaged to obtain the MSCS score. A decrease in MSCS score reflected an improvement in symptoms.

TOS is a measure of symptom response to treatment. At 4 hours and 24 hours, patient assessment of response characterized by their change from baseline in symptom severity and collected by anatomic site of attack involvement, was recorded on a categorical scale (significant improvement [100], improvement [50], same [0], worsening [-50], significant worsening [-100]). The response at each anatomic site was weighted by baseline severity and then the weighted scores across all involved sites were averaged to calculate the TOS. A TOS value >0 reflected an improvement in symptoms from baseline.

#### **EDEMA4**

EDEMA4 was a randomized, double-blind, placebo-controlled trial in which 96 patients were randomized 1:1 to receive KALBITOR 30 mg subcutaneous or placebo for acute attacks of HAE. The primary endpoint was the change from baseline in MSCS score at 4 hours, and the TOS at 4 hours was a key secondary endpoint. Patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients with placebo and the results were statistically significant (Table 2). At 24 hours, patients treated with KALBITOR also demonstrated a greater decrease from baseline in the MSCS than placebo (-1.5 vs. -1.1; p = 0.04) and a greater TOS (89 vs. 55, p = 0.03).

Table 2: Change in MSCS Score and TOS at 4 Hours

	EDEMA4		EDEMA3	
	KALBITOR (N=48)	Placebo (N=48)	KALBITOR (N=36)	Placebo (N=36)
Change in MSCS	Score at 4 Hours		_	
n	47	42	34	35
Mean	-0.8	-0.4	-1.1	-0.6
95% CI	-1.0, -0.6	-0.6, -0.1	-1.4, -0.8,	-0.8, -0.4
P-value	0.010		0.041	
OS at 4 Hours				
n	47	42	34	35
Mean	53	8	63	36
95% CI	39, 68	-12, 28	49, 76	17, 54
P-value	0.003		0.04	

MSCS: Mean Symptom Complex Severity

TOS: Treatment Outcome Score

CI: confidence interval

More patients in the placebo group (24/48, 50%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (16/48, 33%).

Some patients reported improvement following a second 30 mg subcutaneous dose of KALBITOR, administered within 24 hours following the initial dose for symptom persistence or relapse, but efficacy was not systematically assessed for the second dose.

#### **EDEMA3**

EDEMA3 was a randomized, double-blind, placebo-controlled trial in which 72 patients were randomized 1:1 to receive KALBITOR or placebo for acute attacks of HAE. EDEMA3 was similar in design to EDEMA4 with the exception of the order of the prespecified efficacy endpoints. In EDEMA3, the primary endpoint was the TOS at 4 hours, and the key secondary efficacy endpoint was the change from baseline in MSCS at 4 hours. As in EDEMA4, patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients treated with placebo and the results were statistically significant (Table 2).

In addition, more patients in the placebo group (13/36, 36%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (5/36, 14%).

# 16 HOW SUPPLIED/STORAGE AND HANDLING

KALBITOR (ecallantide) is supplied as three 10 mg/mL single-use vials packaged in a carton. Each vial contains 10 mg of ecallantide. Each vial contains a slight overfill.

• NDC (47783-101-01): 3 single-use vials in 1 carton

KALBITOR should be kept refrigerated (2°C to 8°C/36°F to 46°F). Vials removed from refrigeration should be stored below 86°F/30°C and used within 14 days or returned to refrigeration until use.

Protect vials from light until use.

Do not use beyond the expiration date.

# 17 PATIENT COUNSELING INFORMATION

See Medication Guide

- Patients should be advised that KALBITOR may cause anaphylaxis and other hypersensitivity reactions. Patients should be advised that KALBITOR should be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Patients who have known clinical hypersensitivity to KALBITOR should be instructed not to receive additional doses of KALBITOR. [see Boxed Warning, Contraindications (4), and Warnings and Precautions (5.1)]
- Patients should be advised to consult the Medication Guide for additional information regarding the risk of anaphylaxis and other hypersensitivity reactions.



## KALBITOR® (KAL-bi-tor)

### (ecallantide)

Read this Medication Guide before you start receiving KALBITOR and before each treatment. There may be new information. This Medication Guide does not take the place of talking to your doctor about your medical condition or your treatment.

What is the most important information that I should know about KALBITOR? Serious allergic reactions may happen in some people who receive KALBITOR. These allergic reactions can be life-threatening and usually happen within 1 hour after receiving KALBITOR.

- KALBITOR should be given to you by a doctor or nurse in a healthcare setting
  where serious allergic reactions and hereditary angioedema (HAE) can be treated.
- Symptoms of a serious allergic reaction to KALBITOR can be similar to the symptoms of HAE, the condition that you are being treated for. Your doctor or nurse should watch you for any signs of a serious allergic reaction after treatment with KALBITOR.
- Tell your doctor or nurse right away if you have any of these symptoms of a serious allergic reaction during or after treatment with KALBITOR:
  - wheezing, shortness of breath, cough, chest tightness, or trouble breathing
  - dizziness, fainting, fast or weak heartbeat, or feeling nervous
  - reddening of the face, itching, hives, or feeling warm
  - swelling of the throat or tongue, throat tightness, hoarse voice, or trouble swallowing
  - runny nose or sneezing

### What is KALBITOR?

KALBITOR is a prescription medicine used to treat sudden attacks of hereditary angioedema (HAE).

KALBITOR is not a cure for HAE.

It is not known if KALBITOR is safe and effective in children under 16 years of age.

### Who should not receive KALBITOR?

Do not receive KALBITOR if you are allergic to KALBITOR.

## What should I tell my doctor before I receive KALBITOR?

Before receiving KALBITOR, tell your doctor if you:

- have ever had an allergic reaction to KALBITOR. See "Who should not take KALBITOR?"
- are pregnant or plan to become pregnant. It is not known if KALBITOR will harm your unborn baby.
- are breast-feeding or plan to breast-feed. It is not known if KALBITOR passes into your breast milk.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list of them to show to your doctor and pharmacist when you get a new medicine.

### How will I receive KALBITOR?

For each dose, you will receive 3 injections just under the skin (subcutaneous or SC injections) of your abdomen, thigh, or upper arm.

### What are the possible side effects?

KALBITOR can cause serious allergic reactions. See "What is the most important information I should know about KALBITOR?").

Common side effects of KALBITOR include:

- · headache
- nausea
- diarrhea
- fever
- injection site reactions, such as redness, rash, swelling, itching, or bruising
- stuffy nose

Call your doctor for advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

### General information about KALBITOR

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. This Medication Guide gives you the most important information about KALBITOR. If you would like more information, talk with your doctor. You can ask your pharmacist or doctor for information about KALBITOR that is written for health professionals.

## What are the ingredients of KALBITOR?

Active Ingredient: ecallantide

Inactive ingredients: disodium hydrogen orthophosphate (dihydrate), monopotassium phosphate, potassium chloride, sodium chloride in water for injection.

Manufactured for: Dyax Corp.

300 Technology Square, Cambridge, MA 02139

**Issued December 2009** 

This Medication Guide has been approved by the U.S. Food and Drug Administration.

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In re U.S. Patent No.: 7,276,480

Attorney Docket No.: D2033-, 312 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment C

U.S. Patent No.: 7,276,480 B1

## ATTACHMEN C



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(45) Date of Patent:

\*Oct. 2, 2007

# (54) PREVENTION AND REDUCTION OF BLOOD

(75) Inventors: Robert C. Ladner, Ijamsville, MD (US); Arthur C. Ley, Newton, MA (US); Shirish Hirani, Arlington, MA (US); Anthony Williams, Melrose, MA

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(\*) Notice:

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(51) Int. CL A61K 38/16

(2006.01)

CO7K 14/00 (2006.01)(52) U.S. Cl. .....

... 514/12; 530/324 (58) Field of Classification Search .... 514/12; 530/324

See application file for complete search history.

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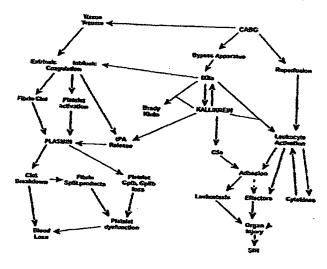
Primary Examiner-Maryam Monshipouri Assistant Examiner-Marsha Tsay (74) Attorney, Agent, or Firm-Fish & Richardson P.C.

(57)

**ABSTRACT** 

Methods are described for preventing or reducing ischemia and/or systemic inflammatory response in a patient such as perioperative blood loss and/or systemic inflammatory response in a patient subjected to cardiothoracic surgery, e.g. coronary artery bypass grafting and other surgical procedures, especially when such procedures involve extra-corporeal circulation, such as cardiopulmonary bypass.

### 4 Claims, 4 Drawing Sheets



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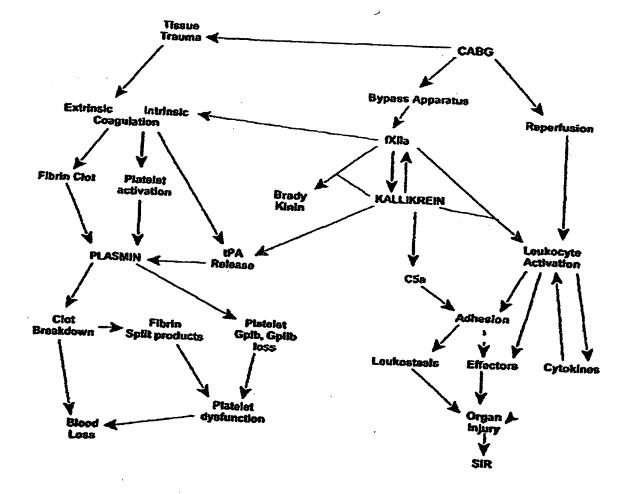
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South

Figure 1



3'AOXI

SAOXI BstB I CG ACT TIT AAC GAC AAC TTG AGA AGA TCA AAA AAC AAC TAA TTA TTC GAA ATG AGA TTC CCA TCT ATC TTC ACT GCT GTT TTG TTC GCT GCT ACG F P s I F T A Y TCC TCT GCT TTG GCT GCT CCA GTT AAC ACC ACT ACT GAA GAC GAG ACT A A P V N T SCT CAA ATT CCT GCT GAG GCT GTC ATC GGT TAC TCT GAC TTG GAA GGT A E V I G Y S D GAC TTC GAC GTC GCT GTT TTG CCA TTC TCT AAC TCT ACT AAC AAC GGT A V L P \_\$ N ຣ TTG TTG TTC ATC AAC ACT ACC ATC GCT TCT ATC GCT GCT-AAG GAG GAA I N T T I A GET GIT TOO CITC GAG AAG AGE GAG GOT ATG CAC JCT TIC TGT GOT TIC A M H A AAG GCT GAC GAC GGT OCG TGC AGA GCT GCT CAC CCA AGA TGG TTC TTC P C R A A H P R F AAC ATC TTC ACG CGT CAA TGC GAG GAG TTC ATC TAC GGT GGT TGT GAG C E E F I GGT AAC CAA AAC AGA TTC GAG TCT CTA GAG GAG TGT AAG AAG ATG TGT F E L E E Ecor I ACT AGA GAC TAG TAG GAA TTC GCC TTA GAC ATG ACT GTT CCT CAG TTC ¥

AAG TTG GGC ACT TAC GAG AAG
3'AOXI

Oct. 2, 2007

#### FIGURE 3A

```
SEQ ID 2:
              (amino acids 3-60) ----MHSFCAPKA-DDGPCRAAHPRWPFNIFTRQCEEFIYGG
   SEQ ID 4:
                                 ----MHSFCAPKA-DDGPCKANHLRFFFNIFTRQCEBFSYGG
   SEQ ID 5:
                                 ---- MHSFCAPKA-DOGHCKANHQRFFFNIFTRQCEEFTYGG
   SEQ ID 6:
                                 ----mhspcapka-ddghckanhqrfpfniftrqcbqftygg
   SEQ ID 7:
                                 ----MHSFCAFKA-DDGHCKASLPRFFFN1FTRQCBEF1YGG
  SEQ ID 8:
                                ----mhsfcafka-ddghckanhorfffniftrocebfsygg
  SEQ ID 9:
                                ----mhspcakfa-ddghckgahlrfpfniftrqcebpiygg
  SEQ ID 10:
                                ----mhspcapka-ddgrckgahlrfffniftrqcebfiygg
  SEQ ID 11:
                                ----MHSFCAPKA-DGGRCRGAHPRWFFNIFTRQCEEFSYGG
  SEQ ID 12:
                                ----MHSFCAPKA-DDGPCRAAHPRWFFNIFTRQCEEPSYGG
  SEQ ID 13:
                                ----mhspcafka-dvgrcrgahprwffniftroceefsygg
  SEQ ID 14:
                                ----MHSFCAFKA-DVGRCRGAQPRFFFN1FTRQCEEFSYGG
  SEQ ID 15:
                                ----mhsfcafka-ddgscraahlrwffniftrqcbefsygg
  SEQ ID 16:
                                ----mhsfcafka-eggscraahqrwffniftrqcbefsygg
  SEQ ID 17:
                                ----mhsfcafka-ddgpcrgahlrfffniftrqceefsygg
  SEQ ID 18:
                                ----mhspcafka-ddghcrgalprnffniftrqceepsygg
  SEQ ID 19:
                                ----MHSFCAFKA-DSGNCRGNLPRFFFNIFTRQCBEFSYGG
  SEQ ID 20:
                                ----MHSFCAFKA-DSGRCRGNHQRFFFN1FTRQCEEFSYGG
  SEQ ID 21:
                                ----mhspcapka-dggrcraiqprwffniftrqcbefsygg
  SEQ ID 22:
                                ----mhspcafka-ddgrcrgahprwffniftrqceefsygg
  BPTI (SEQ ID 29):
                               ---- RPDFCLEPP-YTGPCKARIIRYFYNAKAGLCQTFVYGG
 ITI-D1 (SEQ ID 30):
                               ----KEDSCOLGY-SAGPCMGMTSRYFYNGTSMACETFQYGG
 ITI-D2 (SEQ ID 31):
                               ----TVAACNLPI-VRGPCRAFIQLWAFDAVKGKCVLFPYGG
 LACI-D1 (SEQ ID 32):
                               ----mhspcapka-ddgpckaimkrfpfniftrqceefiygg
 LACI-D2 (SEQ ID 33):
                               ----KPDFCFLEE-DPGICRGYITRYFYNNQTKQCERFKYGG
 LACI-D3 (SEQ ID 34):
                               ----GPSWCLTPA-DRGLCRANENRFYYNSVIGKCRPPKYSG
 HKI B9 (SEQ ID 35):
                               ----LPNVCAFPM-EKGPCQTYMTRWPFNFETGECBLPAYGG
 C=3 (SEQ ID 36):
                              ----ETDICKLPK-DEGTCRDFILKWYYDPNTKSCARFWYGG
 TFPI-2 D1 (SEQ ID 37):
                               ----naeicllpl-dygpcralllryyydrytoscroflygg
 TFPI-2 D2 (SEQ ID 38):
                               ----VPKVCRLQVSVDDQCEGSTEKYFFNLSSMTCEKFFSGG
 TFPI-2 D3 (SEQ ID 39):
                              ----IPSFCYSPK-DEGLCSANVTRYYFNPRYRTCDAFTYTG
 APP-I (SEQ ID 40):
                              ---rnrevcseqa-etgpcramisrwyfdvtegkcappfygg
EpiNE7 (SEQ ID 41):
                              ----RPDFCLEPP-YTGPCVAMPPRYFYNAKAGLCQTFVYGG
BITI-E7-141 (SEQ ID 42):
                              ----RPDFCQLGY-SAGPCVAMPPRYFYNGTSMACQTFVYGG
MUTT26A (SEQ ID 43):
                              ---- RPDFCQLGY-SAGPCVAMPPRYFYNGASMACQTFVYGG
MUTQE (SEQ ID 44):
                              ----RPDFCQLGY-SAGPCVAMFPRYFYNGTSMACETFVYGG
MUT1619 (SEQ ID 45):
                              ----RPDFCQLGY-SAGPCVGMFSRYFYNGTSMACQTFVYGG
EPI-HNE-1 (SEQ ID 46):
                              EAEARPDFCLEPP-YTGPCIAFFPRYFYNAKAGLCOTFVYGG
EPI-HNE-2 (SEQ ID 47):
                              -----Aacnlpi-vrgpciaffprwapdavkgkcvlppygg
EPI-HNE-3 (SEQ ID 48):
                              -----aacnlpi-vrgpciaffprwafdavkgkcvlppygg
EPI-HNE-4 (SEQ ID 49):
                              -----Bacnlpi-vrgpciapfprwafdavkgkcvlfpygg
DPI14 KR (SEQ ID 50):
                              -- Eavrevcseqa-etgpciaffprwyfdvtegkcapfpygg
DPI24 KR (SEQ ID 51):
                              -- Eanae I Cllpl-dygpciaffpryyydryt Qscroflygg
DPI68 KR (SEQ ID 52):
                              -- EAKPOFCFLEE-DPGICIGFFPRYFYNNQAKQCERFVYGG
DPI84 KR (SEQ ID 53):
                              -- EAETDICKLPK-DEGTCIAFFPRWYYDPNTKSCARFVYGG
```

### FIGURE 3B

SEQ ID 2: (cont.)	CEGNONRFBSLEECKKMCTRD
SEQ ID 4: (cont.)	CGGNQNRPESLEECKKMCTRD
SEQ ID 5: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 6: (cont.)	CAGNO NRFESLBECKKMCTRD
SEQ ID 7: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 8: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 9: (cont.)	CEGNQNRFESLEECKKMCTRD
SEQ ID 10: (cont.)	CEGNQNRFESLEECKKMCTRD
SEQ ID 11: (cont.) SEQ ID 12: (cont.)	CGGNQNRFESLEECKKMCTRD
	CGGNQNRFESLEECKKMCTRD
	CGGNQNRFESLBECKKMCTRD
• • • •	CGGNONRFESLEECKKMCTRD
· · · · · · · · · · · · · · · · · · ·	CGGNQNRFESLEECKKMCTRD
SEQ ID 16: (cont.) SEQ ID 17: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 18: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 19: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 20: (cont.)	CGGNQNRPESLEECKKMCTRD
SEQ ID 21: (cont.)	CGGNQNRFESLEECKKMCTRD
SEQ ID 22: (cont.)	CGGNQNRFESLEECKKMCTRD
BPTI (SEQ ID 29): (cont.)	CGGNQNRFESLEECKKMCTRD
ITI-D1 (SEQ ID 30): (cont.)	CRAKRNNFKSAEDCMRTCGGA
ITI-D2 (SEQ ID 31): (cont.)	CMGNGNNFVTEKBCLQTCRTV
LACI-D1 (SEQ ID 32): (cont.)	CQGNGNKFYSEKBCREYCGVP
LACI-D2 (SEQ ID 33): (cont.)	CEGNQNRFESLEECKKMCTRD
LACI-D3 (SEQ ID 34): (cont.)	CLGNMNNFETLEECKNICEDG
HKI B9 (SEQ ID 35): (cont.)	CGGNENNFTSKQECLRACKKG
C≈3 (SEQ ID 36): (cont.)	CGGNS NNFLRKBKCEKFCKFT
TFPI-2 D1 (SEQ ID 37): (cont.)	CGGNENRPGSQKECEKVCAPV
TFPI-2 D2 (SEQ ID 38): (cont.)	Cegnannfytweacddacwri Chrnrienrfpdeatcmgfcapk
TFPI-2 D3 (SEQ ID 39): (cont.)	CGGNDNNFVSREDCKRACAKA
APP-I (SEQ ID 40): (cont.)	CGGNRNNFDTEBYCMAVCGSA
EpiNB7 (SEQ ID 41): (cont.)	CMGNGNNFKSAEDCMRTCGGA
BITI-E7-141 (SEQ ID 42): (cont.)	CMGNGNNFVTEKDCLQTCRGA
MUTT26A (SEQ ID 43): (cont.)	CMGNGNNPVTEKDCLOTCRGA
MUTQE (SEQ ID 44): (cont.)	CMGNGNNFVTEKDCLQTCRGA
MUT1619 (SEQ ID 45): (cont.)	CMGNGNNFVTEKDCLQTCRGA
EPI-HNE-1 (SEQ ID 46): (cont.)	CMGNGNNPKSAEDCMRTCGGA
EPI-HNE-2 (SEQ ID 47): (cont.)	CQGNGNKPYSEKECREYCGVP
BPI-HNE-3 (SEQ ID 48): (cont.)	CQGNGNKFYSEKECREYCGVP
BPI-HNE-4 (SEQ ID 49): (cont.)	COGNGNKFYSEKECREYCGVP
DPI14 KR (SEQ ID 50): (cont.)	CGGNR NNFDTEEY CMAVCGSA
DPI24 KR (SEQ ID 51): (cont.)	CEGNANNFYTWEACDDACWRI
DP168 KR (SEQ ID 52): (cont.)	CLGNMNNFETLEECKNICEDG
DPI84 KR (SEQ ID 53): (cont.)	CGGNENKFGSQKECEKVCAPV
	AAKINDONIACULA

## PREVENTION AND REDUCTION OF BLOOD LOSS

### RELATED APPLICATION

This application claims the benefit of U.S. application Ser. No. 10/456,986, filed Jun. 6, 2003, now U.S. Pat. No. 7,064,107, which claims the benefit from U.S. Provisional Application No. 60/387,239, filed Jun. 7, 2002, and U.S. Provisional Application No. 60/407,003, filed Aug. 28, 10 2002.

The entire teachings of the above applications are incorporated herein by reference.

### BACKGROUND OF THE INVENTION

Proteases are involved in a broad range of biological pathways. In particular, serine proteases such as kallikrein, plasmin, elastase, urokinase plasminogen activator, thrombin, human lipoprotein-associated coagulation inhibitor, and 20 coagulation factors such as factors VIIa, IXa, Xa, XIa, and XIIa have been implicated in pathways affecting blood flow, e.g., general and focal ischemia, tumor invasion, fibrinolysis, perioperative blood loss, and inflammation. Inhibitors of specific serine proteases, therefore, have received attention 25 as potential drug targets for various ischemic maladies.

One such inhibitor, aprotinin (also called bovine pancreatic trypsin inhibitor or BPTI), obtained from bovine hing, has been approved in the United States for prophylactic use in reducing perioperative blood loss and the need for transfusion in patients undergoing cardiopulmonary bypass (CPB), e.g., in the course of a coronary artery bypass grafting procedure. Aprotinin is commercially available under the trade name TRASYLOL® (Bayer Corporation Pharmaceutical Division, West Haven, Conn.) and was 35 previously approved for use to treat pancreatitis. The effectiveness of aprotinin is associated with its relatively nonspecific abilities to inhibit a variety of serine proteases, including plasma kallikrein and plasmin. These proteases are important in a number of pathways of the contact 40 activation system (CAS).

CAS is initially activated when whole blood contacts the surface of foreign substrates (e.g., kaolin, glass, dextran sulfate, or damaged bone surfaces). Kallikrein, a serine protease, is a plasma enzyme that initiates the CAS cascade 45 leading to activation of neutrophils, plasmin, coagulation, and various kinins. Kallikrein is secreted as a zymogen (pre-kallikrein) that circulates as an inactive molecule until activated by a proteolytic event early in the contact activation cascade. Clearly, specific inhibition of kallikrein would 50 be a very attractive approach to control blood loss associated with CPB and the onset of systemic inflammatory response (SIR) as would be encountered during, for example, various invasive surgical procedures.

Despite being the only licensed compound for preventing 55 perioperative blood loss in CPB for coronary artery bypass grafting (CABG) procedures, aprotinin is not as widely used as would be expected. There are serious concerns regarding the use of this bovine polypeptide in patients who require CPB, and in particular the use of this compound in CABG 60 procedures. Aprotinin is not specific for kallikrein, but interacts with additional enzymes (e.g., plasmin) in multiple pathways. Thus, the mechanism of action of aprotinin is largely speculative, and the lack of precise understanding of what is affected during aprotinin treatment produces the risk 65 of complications during treatment. One frequently cited complication is uncontrolled thrombosis, due to aprotinin's

actions upon the fibrinolytic pathway. There is concern not only over such hyperacute events as major vessel thrombosis in the perioperative period, but also over graft patency after the CABG procedure. Furthermore, as a naturally occurring protein obtained from bovine lung, administration of aprotinin in humans can elicit severe hypersensitivity or anaphylactic or anaphylactoid reactions after the first and, more often, after repeat administration to patients. This is particularly of concern in the large number of patients who have repeat CABG procedures. In addition, there is an increasing public concern regarding use of material derived from bovine sources as a potential vector for the transmission of bovine spongiform encephalopathy to humans.

These concerns make clear that a need remains for more 15 effective and more specific means and methods for preventing or reducing perioperative blood loss and the onset of SIR in a patient subjected to surgery resulting in activation of the CAS, such as CABG procedures in patients of CPB, or hip replacement.

### SUMMARY OF THE INVENTION

This invention is based on the discovery of peptides that inhibit serine proteases. Serine proteases such as, for example, kallikrein, are involved in, for example, pathways leading to excessive perioperative blood loss and the onset of systemic inflammatory response. Preferred kallikrein peptide inhibitors include those described in U.S. Pat. Nos. 6,333,402 and 6,057,287 to Markland et al., the contents of which are incorporated herein by reference in their entirety. The invention is directed in part to the use of the peptides in therapeutic methods and compositions suitable for use in eliminating or reducing various ischemias, including but not limited to perioperative blood loss, and the onset of systemic inflammatory response. Perioperative blood loss results from invasive surgical procedures that lead to contact activation of complement components and the coagulation/ fibrinolysis systems. More specifically, the invention provides methods of using kallikrein inhibitors to reduce or prevent perioperative blood loss and a systemic inflammatory response in patients subjected to invasive surgical procedures, especially cardiothoracic surgeries.

In one embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide comprising the amino acid sequence: Xaal Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1), wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa56, Xaa57 or Xaa58 are each individually an amino acid or absent; Xaa10 is an amino acid selected from the group consisting of: Asp and Glu; Xaall is an amino acid selected from the group consisting of: Asp, Gly, Ser, Val, Asn, Ile, Ala and Thr, Xaa13 is an amino acid selected from the group consisting of: Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys and Gln; Xaa15 is an amino acid selected from the group consisting of Arg, Lys, Ala, Ser, Gly, Met, Asn and Gln; Xaa16 is an amino acid selected from the group consisting of: Ala, Gly, Ser, Asp and Asn; Xaal7 is an amino acid selected from the group consisting of: Ala, Asn, Ser, Ile, Gly, Val, Gln and Thr; Xaa18 is an amino acid selected from the group consisting of: His, Leu, Gln and Ala; Xaa19 is an amino acid selected from the group

100 500

consisting of: Pro, Gln, Leu, Asn and Ile; Xaa21 is an amino acid selected from the group consisting of: Trp, Phe, Tyr, His and lle; Xaa22 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa23 is an amino acid selected from the group consisting of: Tyr and Phe, Xaa31 is an 5 amino acid selected from the group consisting of: Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile and Thr, Xaa32 is an amino acid selected from the group consisting of: Glu, Gln, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly and Val; Xaa34 is an amino acid selected from the group consisting of: Thr, Ile, Ser, Val, 10 Ala, Asn, Gly and Leu; Xaa35 is an amino acid selected from the group consisting of: Tyr, Trp and Phe; Xaa39 is an amino acid selected from the group consisting of: Glu, Gly, Ala, Ser and Asp; Xaa40 is an amino acid selected from the group consisting of: Gly and Ala; Xaa43 is an amino acid 15 selected from the group consisting of: Asn and Gly; Xaa45 is an amino acid selected from the group consisting of: Phe and Tyr; and wherein the polypeptide inhibits kallikrein.

In a particular embodiment, the ischemia is perioperative blood loss due to a surgical procedure performed on the 20 patient. The surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In a particular embodiment, individual amino acid posi-Xaal0 is Asp, Xaal1 is Asp, Xaal3 is Pro, Xaal5 is Arg, Xaal6 is Ala, Xaal7 is Ala, Xaal8 is His, Xaal9 is Pro, Xaa21 is Trp, Xaa31 is Glu, Xaa32 is Glu, Xaa34 is Ile, Xaa35 is Tyr, Xaa39 is Glu.

In another embodiment, the invention is directed to a 30 method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide comprising the amino acid sequence: Xaal Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 35 Xaa9 Xaa10 Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa5 Cys Xaa52 40 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1), wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa56, Xaa57 or Xaa58 are each individually an amino acid or absent; Xaal0 is an amino acid selected from the group consisting of: Asp and Glu; Xaall is an amino acid selected from the group 45 consisting of Asp, Gly, Ser, Val, Asn, Ile, Ala and Thr; Xaal3 is an amino acid selected from the group consisting of: Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys and Gin; Xaa15 is an amino acid selected from the group consisting of: Arg, Lys, Ala, Ser, Gly, Met, Asn and Gin; Xaa16 is an 50 amino acid selected from the group consisting of: Ala, Gly, Ser, Asp and Asn; Xaa17 is an amino acid selected from the group consisting of: Ala, Asn, Ser, Ile, Gly, Val, Gin and Thr; Xaa18 is an amino acid selected from the group consisting of: His, Leu, Gin and Ala; Xaa19 is an amino acid selected ss from the group consisting of: Pro, Gin, Leu, Asn and Ile; Xaa21 is an amino acid selected from the group consisting of: Trp, Phe, Tyr, His and Ile; Xaa22 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa23 is an amino acid selected from the group consisting of: Tyr 60 and Phe; Xaa31 is an amino acid selected from the group consisting of: Glu, Asp, Gin, Asn, Ser, Ala, Val, Leu, Ile and Thr, Xaa32 is an amino acid selected from the group consisting of: Glu, Gin, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly and Val; Xaa34 is an amino acid selected from the group 65 consisting of: Thr, Ile, Ser, Val, Ala, Asn, Gly and Leu; Xaa35 is an amino acid selected from the group consisting

of: Tyr, Trp and Phe; Xaa39 is an amino acid selected from the group consisting of: Glu, Gly, Ala, Ser and Asp; Xaa40 is an amino acid selected from the group consisting of: Gly and Ala; Xaa43 is an amino acid selected from the group consisting of: Asn and Gly, Xaa45 is an amino acid selected from the group consisting of: Phe and Tyr; and wherein the polypeptide inhibits kallikrein. In a particular embodiment, the surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting. In a particular embodiment, individual amino acid positions of SEQ ID NO:1 can be one or more of the following: Xaa10 is Asp, Xaa11 is Asp, Xaa13 is Pro, Xaal5 is Arg, Xaal6 is Ala, Xaal7 is Ala, Xaal8 is His, Xaa19 is Pro, Xaa21 is Trp, Xaa31 is Glu, Xaa32 is Glu, Xaa34 is Ile, Xaa35 is Tyr, Xaa39 is Glu.

In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gln Cys Glu Glu Phe lle Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys tions of SEQ ID NO:1 can be one or more of the following: 25 Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In another embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gin Asn Arg Phe Giu Ser Leu Giu Giu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In a particular embodiment, the ischemia can be perioperative blood loss due to a surgical procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Giu Gly Asa Gin Asa Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

In another embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe lle Tyr Gly Gly Cys Glu Gly Asn Gin Asn Arg Phe Ghu Ser Leu Ghu Ghu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In a particular embodiment,

the ischemia can be perioperative blood loss due to a surgical procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a simplified diagram of major multiple pathways and related events involved in the contact activation system 10 commonly the heart and lungs. Typical diseases treated by and systemic inflammatory response (SIR) that can arise in a patient subjected to soft and bone tissue trauma such as that associated with a coronary artery bypass grafting (CABG) procedure, especially when the CABG procedure involves extra-corporeal blood circulation, such as cardiop- 15 ulmonary bypass (Bypass Apparatus). Arrows indicate activation from one component or event to another component or event in the cascade. Arrows in both directions indicate activating effects of components or events in both directions. Broken arrows indicate likely participation of one compo- 20 nent or event in the activation of another component or event. Abbreviations are as follows: "tPA"=tissue plasminogen activator, "C5a"=a protein component of the complement system; "IXIIa" =activator protein of prekallikrein to form active kallikrein; "Extrinsic" extrinsic coagulation 25 system; "Intrinsic"=intrinsic coagulation system.

FIG. 2 shows a portion of a DNA and corresponding deduced amino acid for a KI polypeptide of the invention in plasmid pPIC-K503. The inserted DNA encodes the mat.alpha. prepro signal peptide of Saccharomyces cerevisiae 30 (underlined) fused in frame to the amino terminus of the PEP-1 KI polypeptide having the amino acid sequence enclosed by the boxed area. The amino acid sequence of the PEP-1 KI polypeptide shown in the boxed region is SEQ ID NO:2, and the corresponding nucleotide coding sequence of 35 the KI polypeptide is SEQ ID NO:3. The dashed arrows indicate the location and direction of two PCR primer sequences in AOX regions that were used to produce sequencing templates. DNA sequence for the entire nucleotide sequence of the figure comprises the structural coding 40 sequence for the fusion protein and is designated SEQ ID NO:27. The entire amino acid sequence is SEQ ID NO:28. The double underlined portion of the sequence indicates a diagnostic probe sequence. BstBI and EcoRI indicate locations of their respective palindromic, hexameric, restriction 45 endonuclease sites in the sequence. Asterisks denote translational stop codons.

FIGS. 3A and 3B show an alignment of amino acid sequences of the preferred embodiments of the invention, the native LACI sequence from which these variants were 50 derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOS:29-31 and 33-53). Cysteine residues are highlighted.

### DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention

The invention is based on the discovery of a group of 60 kallikrein inhibitor (KI) polypeptides that inhibit plasma kallikrein with a specificity that permits their use in improved methods of preventing or reducing ischemia such as, for example, perioperative blood loss and/or a systemic inflammatory response (SIR) induced by kallikrein, especially, for example, in patients undergoing surgical procedures and particularly surgical procedures involving cardio-

thoracic surgery, e.g., cardiopulmonary bypass (CPB), such as a coronary artery bypass graft (CABG) procedures. K's can be used specifically for, e.g., pediatric cardiac surgery, lung transplantation, total hip replacement and orthotopic liver transplantation, and to reduce or prevent perioperative stroke during CABG, extracorporeal membrane oxygenation (ECMO) and cerebrovascular accidents (CVA) during these procedures.

Cardiothoracic surgery is surgery of the chest area, most cardiothoracic surgery include coronary artery disease, tumors and cancers of the lung, esophagus and chest wall, heart vessel and valve abnormalities, and birth defects involving the chest or heart. Where cardiothoracic surgery is utilized for treatment, the risk of blood loss (e.g., surgeryinduced ischemia) and the onset of a systemic inflammatory response (SIR) is incurred. Surgery-induced SIR can result in severe organ dysfunction (systemic inflammatory response syndrome; SIRS).

Polypeptides Useful in the Invention

KI polypeptides useful in the invention comprise Kunitz domain polypeptides. In one embodiment these Kunitz domains are variant forms of the looped structure comprising Kunitz domain 1 of human lipoprotein-associated coagulation inhibitor (LACI) protein. LACI contains three internal, well-defined, peptide loop structures that are paradigm Kunitz domains (Girard, T. et al., 1989. Nature, 338:518-520). The three Kunitz domains of LACI confer the ability to bind and inhibit kallikrein, although not with exceptional affinity. Variants of Kunitz domain 1 of LACI described herein have been screened, isolated and bind kallikrein with enhanced affinity and specificity (see, for example, U.S. Pat. Nos. 5,795,865 and 6,057,287, incorporated herein by reference). An example of a preferred polypeptide useful in the invention has the amino acid sequence defined by amino acids 3-60 of SEQ ID NO:2.

Every polypeptide useful in the invention binds kallikrein, and preferred polypeptides are also kallikrein inhibitors (KI) as determined using kallikrein binding and inhibition assays known in the art. The enhanced affinity and specificity for kallikrein of the variant Kunitz domain polypeptides described herein provides the basis for their use in cardiothoracic surgery, e.g., CPB and especially CABG surgical procedures, to prevent or reduce perioperative blood loss and/or the onset of SIR in patients undergoing such procedures. The KI polypeptides used in the invention have or comprise the amino acid sequence of a variant Kunitz domain polypeptide originally isolated by screening phage display libraries for the ability to bind kallikrein.

KI polypeptides useful in the methods and compositions of the invention comprise a Kunitz domain polypeptide comprising the amino acid sequence:

Xaa1 Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 55 Xaall Gly Xaal3 Cys Xaal5 Xaal6 Xaal7 Xaal8 Xaal9 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1)

"Xaa" refers to a position in a peptide chain that can be any of a number of different amino acids. For example, for the KI peptides described herein, Xaa10 can be Asp or Gh; Xaall can be Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr, Xaal3 can be Pro, Arg, His, Asn, Ser, Thr, Ala, Gly, Lys or Gln; Xaa15 can be Arg, Lys, Ala, Ser, Gly, Met, Asn or Gln; Xaa16 can be Ala, Gly, Ser, Asp or Asn; Xaa17 can be Ala,

Asn, Ser, Ile, Gly, Val, Gln or Thr; Xaa18 can be His, Leu, Gln or Ala; Xaa19 can be Pro, Gln, Leu, Asn or Ile; Xaa21 can be Trp, Phe, Tyr, His or Ile; Xaa31 can be Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile or Thr; Xaa32 can be Glu, Gln, Asp Asn, Pro, Thr, Leu, Ser, Ala, Gly or Val; Xaa34 can be 5 Ile, Thr, Ser, Val, Ala, Asn, Gly or Leur, Xaa35 can be Tyr, Trp or Phe; Xaa39 can be Glu, Gly, Ala, Ser or Asp. Amino acids Xaa6, Xaa7, Xaa8, Xaa9, Xaa20, Xaa24, Xaa25, Xaa26, Xaa27, Xaa28, Xaa29, Xaa41, Xaa42, Xaa44, four and at last three amino acids of SEQ ID NO: 1 can optionally be present or absent and can be any amino acid, if present.

polypeptides that bind to kallikrein. For example, in a preferred embodiment of the invention, a KI polypeptide useful in the methods and compositions of the invention has the following variable positions: Xaall can be Asp, Gly, Ser or Ile, Xaa18 can be His, Leu or Gin; Xaa19 can be Pro, Gin or Leu; Xaa21 can be Trp or Phe; Xaa31 is Glu; Xaa32 can be Glu or Gln; Xaa34 can be Ile, Thr or Ser; Xaa35 is Tyr; and Xaa39 can be Glu, Gly or Ala.

A more specific embodiment of the claimed invention is defined by the following amino acids at variable positions: Xaal0 is Asp; Xaal1 is Asp; Xaal3 can be Pro or Arg; Xaal 5 is Arg; Xaal 6 can be Ala or Gly; Xaal 7 is Ala; Xaal 8 is His; Xaa19 is Pro; Xaa21 is Trp; Xaa31 is Glu; Xaa32 is 30 Glu; Xaa34 can be Ile or Ser; Xaa35 is Tyr; and Xaa39 is Gly.

Also encompassed within the scope of the invention are peptides that comprise portions of the polypeptides described herein. For example, polypeptides could comprise 35 binding domains for specific kallikrein epitopes. Such fragments of the polypeptides described herein would also be encompassed.

KI polypeptides useful in the methods and compositions described herein comprise a Kunitz domain. A subset of the 40 sequences encompassed by SEQ ID NO:1 are described by the following (where not indicated, "Xaa" refers to the same set of amino acids that are allowed for SEQ ID NO:1):

Met His Ser Phe Cys Ala Phe Lys Ala Xaa10 Xaa11 Gly Xaa34 Xaa35 Gly Gly Cys Xaa39 Gly Asn Gln Asn Arg Phe Ghu Ser Leu Ghu Ghu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:54).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro 50 Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Giu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO.2),

Cys Lys Ala Asn His Leu Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Giu Giu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:4),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His 60 Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Thr Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:5),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His 65 Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Gin Gin Phe Thr Tyr Giy Giy Cys Ala Giy Asn

Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:6),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala Ser Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Giu Giu Phe Ile Tyr Giy Giy Cys Giy Giy Asn Gin Asn Arg Phe Glu Ser Leu Gin Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:7),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala Asn His Gln Arg Phe Phe Phe Asn lie Phe Thr Xaa46, Xaa47, Xaa48, Xaa49, Xaa50, Xaa52, Xaa53 and 10 Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Len Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:8),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Gly Ala His Leu Arg Phe Phe Phe Asu Ile Phe Thr Peptides defined according to SEQ ID NO:1 form a set of 15 Arg Gin Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:9).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg or Val; Xaa13 can be Pro, Arg, His or Asn; Xaa15 can be Arg 20 Arg Gln Cys Glu Glu Phe He Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:10),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr 25 Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:11),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:12),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Giu Ser Leu Giu Giu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:13),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly Ala Gin Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Giu Giu Phe Ser Tyr Giy Giy Cys Giy Giy Asn Gln Asn Arg Phe Glu Ser Leu Glu Gln Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO: 14),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Ser Xaal3 Cys Xaal5 Xaal6 Xaal7 Xaal8 Xaal9 Arg Xaa21 45 Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:15),

Met His Ser Phe Cys Ala Phe Lys Ala Glu Gly Gly Ser Cys Arg Ala Ala His Gin Arg Trp Phe Phe Asu Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:16),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Gly Ala His Leu Arg Phe Phe Phe Asn Ile Phe Thr Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro 55 Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:17),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Arg Gly Ala Leu Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:18).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Asn Cys Arg Gly Asn Leu Pro Arg Phe Phe Phe Asn lle Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:19),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Arg Cys Arg Gly Asn His Gin Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gin Asn Arg Phe Glu Ser Leo Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:20),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Ala lle Gin Pro Arg Trp Phe Phe Asn lle Phe Thr Arg Gin Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:21),

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:22).

FIGS. 3A and 3B provides an amino acid sequence alignment of these sequences, the native LACI sequence from which these variants were derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOS: 29-31 and 33-53),

The KI polypeptides useful in the methods and compositions described herein can be made synthetically using any standard polypeptide synthesis protocol and equipment. For example, the stepwise synthesis of a KI polypeptide described herein can be carried out by the removal of an 25 amino (N) terminal-protecting group from an initial (i.e., carboxy-terminal) amino acid, and coupling thereto of the carboxyl end of the next amino acid in the sequence of the polypeptide. This amino acid is also suitably protected. The carboxyl group of the incoming amino acid can be activated 30 to react with the N-terminus of the bound amino acid by formation into a reactive group such as formation into a carbodiimide, a symmetric acid anhydride, or an "active ester" group such as hydroxybenzotriazole or pentafluorophenyl esters. Preferred solid-phase peptide synthesis 35 methods include the BOC method, which utilizes tertbutyloxycarbonyl as the .alpha.-amino protecting group, and the FMOC method, which utilizes 9-fluorenylmethloxycarbonyl to protect the alpha.-amino of the amino acid residues. Both methods are well known to those of skill in the 40 art (Stewart, J. and Young, J., Solid-Phase Peptide Synthesis (W. H. Freeman Co., San Francisco 1989); Merrifield, J., 1963. Am. Chem. Soc., 85:2149-2154; Bodanszky, M. and Bodanszky, A., The Practice of Peptide Synthesis (Springer-Verlag, New York 1984), the entire teachings of these 45 references is incorporated herein by reference). If desired, additional amino- and/or carboxy-terminal amino acids can be designed into the amino acid sequence and added during polypeptide synthesis.

Alternatively, Kunitz domain polypeptides and KI 50 polypeptides useful in the compositions and methods of the invention can be produced by recombinant methods using any of a number of cells and corresponding expression vectors, including but not limited to bacterial expression vectors, yeast expression vectors, baculovirus expression vectors, mammalian viral expression vectors, and the like. Kunitz domain polypeptides and KI polypeptides useful in the compositions and methods of the invention can also be produced transgenically using nucleic acid molecules comprising a coding sequence for a Kunitz domain or KI 60 herein as "PEP-1") having the amino acid sequence of SEQ polypeptide described herein, wherein the nucleic acid molecule can be integrated into and expressed from the genome of a host animal using transgenic methods available in the art. In some cases, it could be necessary or advantageous to fuse the coding sequence for a Kunitz domain polypeptide or 65 a KI polypeptide comprising the Kunitz domain to another coding sequence in an expression vector to form a fusion

polypeptide that is readily expressed in a host cell. Preferably, the host cell that expresses such a fusion polypeptide also processes the fusion polypeptide to yield a Kunitz domain or KI polypeptide useful in the invention that contains only the desired amino acid sequence. Obviously, if any other amino acid(s) remain attached to the expressed Kunitz domain or KI polypeptide, such additional amino acid(s) should not diminish the kallikrein binding and/or kallikrein inhibitory activity of the Kunitz domain or KI 10 polypeptide so as to preclude use of the polypeptide in the methods or compositions of the invention.

A preferred recombinant expression system for producing KI polypeptides useful in the methods and compositions described herein is a yeast expression vector, which permits a nucleic acid sequence encoding the amino acid sequence for a KI polypeptide or Kunitz domain polypeptide to be linked in the same reading frame with a nucleotide sequence encoding the matalpha. prepro leader peptide sequence of Saccharomyces cerevisiae, which in turn is under the control 20 of an operable yeast promoter. The resulting recombinant yeast expression plasmid can then be transformed by standard methods into the cells of an appropriate, compatible yeast host, which cells are able to express the recombinant protein from the recombinant yeast expression vector. Preferably, a host yeast cell transformed with such a recombinant expression vector is also able to process the fusion protein to provide an active KI polypeptide useful in the methods and compositions of the invention. A preferred yeast host for producing recombinant Kunitz domain polypeptides and KI polypeptides comprising such Kunitz domains is Pichia

As noted above, KI polypeptides that are useful in the methods and compositions described herein can comprise a Kunitz domain polypeptide described herein. Some KI polypeptides can comprise an additional flanking sequence, preferably of one to six amino acids in length, at the amino and/or carboxy-terminal end, provided such additional amino acids do not significantly diminish kallikrein binding affinity or kallikrein inhibition activity so as to preclude use in the methods and compositions described herein. Such additional amino acids can be deliberately added to express a KI polypeptide in a particular recombinant host cell or can be added to provide an additional function, e.g., to provide a peptide to link the KI polypeptide to another molecule or to provide an affinity moiety that facilitates purification of the polypeptide. Preferably, the additional amino acid(s) do not include cysteine, which could interfere with the disulfide bonds of the Kunitz domain.

An example of a preferred Kunitz domain polypeptide useful in the methods and compositions of the invention has the amino acid sequence of residues 3-60 of SEQ ID NO:2. When expressed and processed in a yeast fusion protein expression system (e.g., based on the integrating expression plasmid pHIL-D2), such a Kunitz domain polypeptide retains an additional amino terminal Glu-Ala dipeptide from the fusion with the mat.alpha. prepro leader peptide sequence of S cerevisiae. When secreted from the yeast host cell, most of the leader peptide is processed from the fusion protein to yield a functional KI polypeptide (referred to ID NO:2 (see boxed region in FIG. 2).

Particularly preferred KI polypeptides useful in the methods and compositions described herein have a binding affinity for kallikrein that is on the order of 1000 times higher than that of aprotinin, which is currently approved for use in CABG procedures to reduce blood loss. The surprisingly high binding affinities of such KI polypeptides

described herein indicate that such KI polypeptides exhibit a high degree of specificity for kallikrein to the exclusion of other molecular targets (see Table 1, below). Thus, use of such polypeptides according to the invention reduces much of the speculation as to the possible therapeutic targets in a 5 patient. The lower degree of specificity exhibited by, for example, aprotinin, leads to possible pleiotropic side effects and ambiguity as to its therapeutic mechanism.

The polypeptides defined by, for example, SEQ ID NO:1 contain invariant positions, e.g., positions 5, 14, 30, 51 and 10 55 can be Cys only. Other positions such as, for example, positions 6, 7, 8, 9, 20, 24, 25, 26, 27, 28, 29, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53 and 54 can be any amino acid (including non-naturally occurring amino acids). In a particularly preferred embodiment, one or more amino acids 15 correspond to that of a native sequence (e.g., SEQ ID NO:32, see FIG. 3). In a preferred embodiment, at least one variable position is different from that of the native sequence. In yet another preferred embodiment, the amino acids can each be individually or collectively substituted by a conservative or non-conservative amino acid substitution. Conservative amino acid substitutions replace an amino acid with another amino acid of similar chemical structure and may have no affect on protein function. Non-conservative amino acid substitutions replace an amino acid with another 25 amino acid of dissimilar chemical structure. Examples of conserved amino acid substitutions include, for example, Asn-→Asp, Arg-→Lys and Ser-→Thr. In a preferred embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and/or 21 of these amino acids can be indepen- 30 dentily or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2.

Other positions, for example, positions 10, 11, 13, 15, 16, 17, 18, 19, 21, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45, can be any of a selected set of amino acids. Thus SEQ ID NO:1 35 defines a set of possible sequences. Each member of this set contains, for example, a cysteine at positions 5, 14, 30, 51 and 55, and any one of a specific set of amino acids at positions 10, 11, 13, 15, 16, 17, 18, 19, 221, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45. In a preferred embodiment, 1, 2, 40 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and/or 19 of these amino acids can be independently or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2. The peptide preferably has at least 80%, at least 85%, at least 90% or at least 95% 45 identity to SEQ ID NO:2.

### Methods and Compositions

The present invention is also directed to methods for preventing or reducing ischemia. Preferred in the invention 50 are methods for preventing or reducing perioperative blood loss and/or a systemic inflammatory response (SIR) in a patient, especially associated with cardiothoracic surgery. A method for treatment involves the administration of a KI polypeptide comprising a Kunitz domain. One embodiment 55 of the method involves using a peptide containing an amino acid sequence of SEQ ID NO:1 that has an affinity for kallikrein that is approximately 1000-fold or more higher than that of a broad range serine protease, e.g., aprotinin, which is isolated from bovine lung and currently approved 60 the nucleotide sequence of nucleotides 7-180 of SEQ ID poration Pharmaceutical Division, West Haven, Conn.).

Patients subjected to any of a number of surgical procedures, especially those involving extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB, 65 and/or bone trauma, such as sternal split or hip replacement, are at risk for perioperative blood loss and inflammation.

Contact of a patient's blood with the cut surfaces of bone or of CPB equipment is sufficient to activate one or several undesirable cascade responses, including a contact activation system (CAS), which can lead to extensive perioperative blood loss requiring immediate blood transfusion, as well as a systemic inflammatory response (SIR), which, in turn, can result in permanent damage to tissues and organs. While not desiring to be limited to any particular mechanism or theory, it appears that the blood loss that occurs associated with cardiothoracic surgery, e.g., CPB, as in a CABG procedure, probably results from extensive capillary leakage, which can result in significant loss of blood that must be replaced by immediate blood transfusion.

The methods described herein are useful for preventing or reducing various ischemias including, for example, perioperative blood loss and SIR in a patient subjected to a surgical procedure, and especially wherein the surgical procedure requires extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB. The methods of the 20 invention are particularly useful for preventing or reducing perioperative blood loss and/or SIR in a patient subjected to a CABG procedure requiring CPB or other cardiac surgery.

Preferred compositions for medical use comprise a KI polypeptide described herein. Such compositions useful can further comprise one or more pharmaceutically acceptable buffers, carriers, and excipients, which can provide a desirable feature to the composition including, but not limited to, enhanced administration of the composition to a patient, enhanced circulating half-life of the KI polypeptide of the composition, enhanced compatibility of the composition with patient blood chemistry, enhanced storage of the composition, and/or enhanced efficacy of the composition upon administration to a patient. In addition to a KI polypeptide described herein, compositions can further comprise one or more other pharmaceutically active compounds that provide an additional prophylactic or therapeutic benefit to a patient of an invasive surgical procedure.

Compositions useful in the methods of the invention comprise any of the Kunitz domain polypeptides or KI polypeptides comprising such Kunitz domain polypeptides described herein. Particularly preferred are KI polypeptides comprising a Kunitz domain polypeptide having a 58-amino acid sequence of amino acids 3-60 of SEQ ID NO:2. An example of such a particularly preferred KI polypeptide useful in the methods and compositions of the invention is the PEP-1 KI polypeptide having the 60-amino acid sequence of SEQ ID NO:2. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO.2 is provided in SEQ ID NO:3 (see, e.g., nucleotides 309-488 in FIG. 2). It is understood that based on the known genetic code, the invention also provides degenerate forms of the nucleotide sequence of SEQ ID NO:3 by simply substituting one or more of the known degenerate codons for each amino acid encoded by the nucleotide sequence. Nucleotides 7-180 of SEQ ID NO:3, and degenerate forms thereof, encode the non-naturally occurring Kunitz domain polypeptide having the 58-amino acid sequence of amino acids 3-60 of SEQ ID

Any of a variety of nucleic acid molecules can comprise NO:3, degenerate forms, and portions thereof, including but not limited to, recombinant phage genomes, recombinant mammalian viral vectors, recombinant insect viral vectors, yeast mini chromosomes, and various plasmids. Such plasmids include those used to clone and/or express such nucleotide coding sequences. Expression vectors provide a promoter, which can be operably linked to a particular

nucleotide sequence and an appropriate host cell, which is able to transcribe the particular nucleotide coding sequence into a functional messenger RNA (mRNA) and also translate the mRNA into the corresponding polypeptide. A polypeptide so produced can then be isolated from the host cell. 5 Nucleic acid molecules comprising a nucleic acid sequence encoding a Kunitz domain or KI polypeptide described herein can be made by standard nucleic acid synthesis methods, recombinant DNA methodologies, polymerase chain reaction (PCR) methods, and any combination thereof. 10

Perioperative Blood Loss and Reduced Heart Bloodflow

Due to the many advances in medicine, a number of highly invasive surgical procedures are carried out each day that result in blood loss, or place patients at a high risk for blood loss. Such patients must be carefully monitored to restore and maintain normal blood supply and hemostasis, and they may need blood transfusions. Surgical procedures that involve blood loss include those involving extra-corporeal circulation methods such as cardiothoracic surgery, e.g., CPB. In such methods, a patient's heart is stopped and the circulation, oxygenation, and maintenance of blood volume are carried out artificially using an extra-corporeal circuit and a synthetic membrane oxygenator. These techniques are commonly used during cardiac surgery. Additionally, it is 25 apparent that surgery involving extensive trauma to bone, such as the sternal split necessary in CABG or hip replacement procedures, is also associated with activation of the CAS, which can result in a variety of disruptions in the blood

Atherosclerotic coronary artery disease (CAD) causes a narrowing of the human of one or several of the coronary arteries; this limits the flow of blood to the myocardium (i.e., the heart muscle) and can cause angina, heart failure, and myocardial infarcts. In the end stage of coronary artery 35 atherosclerosis, the coronary circulation can be almost completely occluded, causing life threatening angina or heart failure, with a very high mortality. CABG procedures may be required to bridge the occluded blood vessel and restore blood to the heart; these are potentially life saving. CABG 40 procedures are among the most invasive of surgeries in which one or more healthy veins or arteries are implanted to provide a "bypass" around the occluded area of the diseased vessel. CABG procedures carry with them a small but important perioperative risk, but they are very successful in 45 providing patients with immediate relief from the mortality and morbidity of atherosclerotic cardiovascular disease. Despite these very encouraging results, repeat CABG procedures are frequently necessary, as indicated by a clear increase in the number of patients who eventually undergo 50 second and even third procedures; the perioperative mortality and morbidity seen in primary CABG procedures is increased in these re-do procedures.

There have been improvements in minimally invasive nearly all CABG procedures performed for valvular and/or congenital heart disease, heart transplantation, and major aortic procedures, are still carried out on patients supported by CPB. In CPB, large cannulae are inserted into the great vessels of a patient to permit mechanical pumping and 60 oxygenation of the blood using a membrane oxygenator. The blood is returned to the patient without flowing through the lungs, which are hypoperfused during this procedure. The heart is stopped using a cardioplegic solution, the patient cooled to help prevent brain damage, and the peripheral 65 circulating volume increased by an extracorporeal circuit, i.e., the CPB circuit, which requires "priming" with donor

blood and saline mixtures are used to fill the extracorporeal circuit. CPB has been extensively used in a variety of procedures performed for nearly half a century with successful outcomes. The interaction between artificial surfaces, blood cells, blood proteins, damaged vascular endothelium, and extravascular tissues, such as bone, disturbs hemostasis and frequently activates the CAS, which, as noted above, can result in a variety of disruptions in the blood and vasculature. Such disruption leads to excess perioperative bleeding, which then requires immediate blood transfusion. A consequence of circulating whole blood through an extracorporeal circuit in CPB can also include the systemic inflammatory response (SIR), which is initiated by contact activation of the coagulation and complement systems. Indeed, much of the morbidity and mortality associated with seemingly mechanically successful CPB surgical procedures is the result of the effects of activating coagulation, fibrinolysis, or complement systems. Such activation can damage the pulmonary system, leading to adult respiratory distress syndrome (ARDS), impairment of kidney and splanchnic circulation, and induction of a general coaguiopathy leading to blood loss and the need for transfusions. In addition to the dangers of perioperative blood loss, additional pathologies associated with SIR include neurocognitive deficits, stroke, renal failure, acute myocardial infarct, and cardiac tissue damage.

Blood transfusions also present a significant risk of infection and elevate the cost of CABG or other similar procedures that require CPB. In the absence of any pharmacological intervention, three to seven units of blood must typically be expended on a patient, even with excellent surgical techniques. Accordingly, there is considerable incentive for the development of new and improved pharmacologically effective compounds to reduce or prevent perioperative bleeding and SIR in patients subjected to CPB and CABG procedures.

Administration and Dosing Considerations for KI Polypep-

KI polypeptides described herein can be administered to a patient before, during, and/or after a surgical procedure in a pharmaceutically acceptable composition. The term "pharmaceutically acceptable" composition refers to a non-toxic carrier or excipient that may be administered to a patient, together with a compound of this invention, and wherein the carrier or excipient not destroy the biological or pharmacological activity of the composition. KI polypeptides described herein can be administered locally or systemically by any suitable means for delivery of a kallikrein inhibitory amount of the KI polypeptides to a patient including but not limited to systemic administrations such as, for example, intravenous and inhalation. Parenteral administration is particularly preferred.

For parenteral administration, the polypeptides can be surgical techniques for uncomplicated CAD. However, 55 injected intravenously, intramuscularly, intraperitoneally, or subcutaneously. Intravenous administration is preferred. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Other pharmacentically acceptable carriers include, but are not limited to, sterile water, saline solution, and buffered saline (including buffers like phosphate or acetate), alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, paraffin, etc. Where necessary, the composition can also include a solubilizing agent and a local anaesthetic such as lidocaine to ease pain at the site of the injection, preservatives, stabilizers, wetting agents, emulsifiers, salts, lubricants, etc. as long as they do not react

deleteriously with the active compounds. Similarly, the composition can comprise conventional excipients, e.g., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active 5 compounds. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent in activity 10 units. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade "water for injection" or saline. Where the composition is to be administered by injection, an ampoule of sterile water for injection or saline 15 can be provided so that the ingredients can be mixed prior to administration.

Preferably, the methods of the invention comprise administering a KI polypeptide to a patient as an intravenous infusion according to any approved procedure. Thus, a KI 20 polypeptide described herein can be administered to a patient subjected to a CABG procedure at the times similar to those currently used in approved protocols for administering aprotinin and in an amount necessary to provide a patient with a required number or concentration of kallikrein 25 inhibitory units (KIU). According to the invention, a KI polypeptide described herein can also be administered to a patient in the immediate postoperative period, when bleeding abnormalities can occur as a consequence of downstream effects of SIR. For example, in a procedure involving 30 CPB, a KI polypeptide described herein can be administered to a patient as an initial loading dose, e.g., an effective amount over the course of a convenient time, such as 10 minutes, prior to induction of anesthesia. Then, at induction of anesthesia, a second dose of KI polypeptide can be 35 in plasma is estimated at approximately 500 nM (Silverberg, injected into the CPB priming fluid ("pump prime volume"). The patient can then be placed on a continuous and controlled intravenous infusion dose for the duration of the surgical procedure, and after the procedure if indicated.

Currently there are two regimens approved in the United 40 States for administering aprotinin to a patient undergoing a CABG procedure (see, product label and insert for TRA-SYLOL®, Bayer Corporation Pharmaceutical Division, West Haven, Conn.). One such approved regimen uses a 2 the pump prime volume, and 500,000 KIU per hour of surgery. Another approved regimen uses 1 million KIU intravenous loading dose, 1 million KIU into the pump prime volume, and 250,000 KIU per hour of surgery. As these regimens are based on KIU, the regimens are readily 50 adapted to any KI polypeptide described herein once the specific activity and KIU of a particular KI polypeptide has been determined by standard assays. Owing to the enhanced binding affinity and inhibitory activity in representative KI polypeptides described herein relative to aprotinin, it is 55 expected that such compositions and methods of the invention are likely to require fewer milligrams (mg) per patient to provide a patient with the required number or concentration of KIU.

Several considerations regarding dosing with a KI 60 polypeptide in methods of the invention can be illustrated by way of example with the representative PEP-1 KI polypeptide of the invention having the amino sequence of SEQ ID NO:2 (molecular weight of 7,054 Daltons).

Table 1, below, provides a comparison of the affinity 65 (K.sub.i,app) of the PEP-1 KI polypeptide for kallikrein and eleven other known plasma proteases.

1TABLE 1 Aprotinin Protease Substrate PEP-1 K.sub.i.app (pM) K.sub.i,app (pM) human plasma kallikrein 44 3.0 .times. 10.sup.4 human urine kallikrein>1 .times. 10.sup.8 4.0 times. 10 sup.3 porcine pancreatic kallikrein 2.7 times. 10.sup.7 550 human C1r, activated>2.0 .times. 10.sup.8>1.0 times. 10.sup.7 human C1s, activated>2.0 times. 10.sup.7>1.0 .times. 10.sup.8 human plasma factor XIa 1.0 times. 10.sup.4 ND human plasma factor XIIa>2.0 .times. 10.sup.7>1.0 .times. 10.sup.8 human plasmin 1.4 .times. 10.sup.5 894 human pancreatic trypsin>2 .times. 10.sup.7

ND human pancreatic chymotrypsin>2.0 .times. 10.sup.7 7.3 times. 10.sup.5 human neutrophil elastase>2.0 times. 10.sup.7 1.7 .times. 10.sup.6 human plasma thrombin>2.0 .times. 10.sup.7>1.0 .times. 10.sup.8 ND=not determined

Clearly, the PEP-1 KI polypeptide is highly specific for human plasma kallikrein. Furthermore, the affinity (K.sub.i, app) of PEP-1 for kallikrein is 1000 times higher than the affinity of aprotinin for kallikrein: the K.sub.i,app of PEP-1 for kallikrein is about 44 pM (Table 1), whereas the K.sub.i, app of aprotinin for kallikrein is 30,000 pM. Thus, a dose of PEP-1 could be approximately 1000 times lower than that used for aproximin on a per mole basis. However, consideration of several other factors may provide a more accurate estimation of the dose of PEP-1 required in practice. Such factors include the amount of kallikrein activated during CPB in a particular patient, the concentration of kallikrein required to elicit an SIR, and the bioavailability and pharmacological distribution of PEP-1 in a patient. Nevertheless, use of a KI polypeptide in methods according to the invention and provided in doses currently approved for the use of aprotinin is still expected to provide significant improvements over the current use of the less specific, lower affinity, bovine aprotinin

For example, the total amount of circulating prekallikrein M. et al., "The Contact System and Its Disorders," in Blood: Principles and Practice of Hematology, Handin, R. et al., eds., J B Lippincott Co., Philadelphia, 1995). If all of the prekallikrein were activated, then at least 500 nM of PEP-1 would be required for a stoichiometric inhibition of kallikrein. An individual having 5 liters of plasma would therefore require about 18 mg of PEP-1 to achieve a plasma concentration of 500 nM.

Another factor to consider is the threshold concentration million KIU intravenous loading dose, 2 million KIU into 45 of kallikrein required to induce a SIR in a patient. If the concentration of active kallikrein must be maintained below, e.g., I nM, then owing to its high affinity for kallikrein, PEP-1 offers a significant advantage over aprotinin in the amount of protein that would be required to inhibit SIR. In particular, a concentration of PEP-1 of 1 nM would inhibit 99.6% of kallikrein present at 1 nM (i.e., only 0.4 pM free kallikrein remaining in the blood), whereas, an aprotinin concentration of 1 nM would only inhibit 24.5% of the kallikrein present at 1 nM. For aprotinin to inhibit 99% of the kallikrein at 1 nM, an aprotinin concentration in the plasma of at least 3 mu.M is required (i.e., 3000 times higher concentration than for PEP-1).

For a patient undergoing CPB, an initial clinical dose of PEP-1 can be estimated from a recommended dose regimen of aprotinin (1 times. 10.sup.6 KIU) mentioned above. Aprotinin is reported in a package insert to have as specific inhibitory activity of 7143 KIU/mg determined using a dog blood pressure assay. Therefore, 1 .times. 1 .sup.6 KIU of aprotinin is equivalent to 140 mg of aprotinin (i.e., 1 .times. 10.sup.6 KIU/7143 KIU/mg=140 mg of aprotinin). In a patient having a blood plasma volume of 5 liters, 140 mg corresponds to approximately 4.3 .mu.M aprotinin (molecular weight of aprotinin is 6512 Daltons). The specific activity of aprotinin in the standard inhibitory assay used for PEP-1 is 0.4 KIU/mg of polypeptide. A dose of 140 mg would correspond to a loading dose for aprotinin of 56 KIU (140 mg.times.0.4 KIU/mg=56 KIU). In contrast, since the specific activity of the PEP-1 KI polypeptide is 10 KIU/mg in the standard inhibition assay, a dose of only 5.6 mg of PEP-1 would be required to provide the number of KIUs equivalent to 140 mg of aprotinin. In a patient with a plasma volume of 5 liters, this corresponds to about 160 nM PEP-1 (molecular 10 weight of PEP-1 is 7054 Daltons), although a higher dose of the PEP-1 KI polypeptide can be required if all of the plasma kallikrein (500 nM) is activated and/or if this KI polypeptide is poorly distributed in a patient.

Furthermore, the KI polypeptides can be non-naturally 15 occurring, and they can be produced synthetically or recombinantly, as noted above, thereby avoiding potential contamination of transmissible diseases that can arise during isolation of a protein from a natural animal source, such as in the case of aprotinin, which is isolated from bovine lung. 20 Increasingly important to administrative and public acceptance of a treatment or pharmaceutical composition comprising a polypeptide is the avoidance of possible contamination with and transmission to human patients of various pathological agents. Of particular interest for the safety of 25 proteins isolated from a bovine tissue is the elimination of the possible risk of exposure to viral mediated diseases, bacterial mediated diseases, and, especially, transmissible bovine spongiform encephalonathies.

As variants of the Kunitz domain 1 of the human LACI 30 protein, fewer side effects are expected from administering the KI polypeptides to patients than for aprotinin, which is a bovine protein that is documented to cause anaphylactic and anaphylactoid responses in patients, especially in repeat administrations, such as second time CABG procedures. 35 Additionally, the highly specific binding of the KI polypeptides described herein to kallikrein will effectively limit or eliminate the thrombotic tendencies observed with aprotinin, and reduce the problems observed with graft patency following CABG procedures.

The invention will be further described with reference to the following non-limiting examples. The teachings of all the patents, patent applications and all other publications and websites cited herein are incorporated by reference in their entirety.

### **EXEMPLIFICATION**

#### Example 1

### A Representative KI Polypeptide

A non-naturally occurring, KI polypeptide useful in the compositions and methods of the invention was identified as a kallikrein binding polypeptide displayed on a recombinant 55 phage from a phage display library. PEP-1 has the following amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2). The molecular weight of PEP-1 is 7,054 Daltons.

The mucleotide sequence (SEQ ID NO:3) encoding the PEP-1 amino acid sequence (SEQ ID NO:2), was derived from a peptide that was isolated and sequenced by standard 65 methods determined from the recombinant phage DNA. PEP-1 was produced in amounts useful for further charac-

terization as a recombinant protein in His4.sup.-phenotype host cells of yeast strain *Pichia pastoris*.

#### Example 2

Construction of a Recombinant Plasmid to Express KI Polypeptides

The initial plasmid, pHIL-D2, is ampicillin resistant and contains a wild-type allele of His4 from *P. pastoris*. The final DNA sequence comprising the coding sequence for the mat.alpha. Prepro-PEP-1 fusion protein in the recombinant expression plasmid pPIC-K503 is shown in FIG. 2. The DNA sequence of pHIL-D2 was modified to produce pPIC-K503, as follows:

1. The BstBI site in the 3' AOX1 region of pHIL-D2, located downstream of the His4 gene, was removed by partial restriction digestion, fill-in, and ligation, altering the sequence from TTCGAA (SEQ ID NO:23) to TTCGCGAA (SEQ ID NO:24). This modification was made to facilitate and direct the cloning of the expression cassette into the plasmid.

2. The AatII site bearing the bla gene located downstream of His4 was removed by restriction digestion, fill-in, and ligation modifying the sequence from GACGTC (SEQ ID NO:25) to GACGTACGTC (SEQ ID NO:26). This modification was made to facilitate the cloning of expression cassettes having AatII sites into the plasmid. The DNA encoding PEP-1 was synthesized based on the nucleotide sequence from the original kallikrein-binding display phage and consisted of 450 base pairs (bp). The final DNA sequence of the insert in the pHIL-D2 plasmid is flanked by a 5' AOX1 sequence and a 3' AOX1 sequence (portions of which are shown in FIG. 2) and encode a fusion protein comprising the mat.alpha. prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 KI polypeptide. The signal peptide was added to facilitate the secretion of PEP-1 from the yeast host cells. The oligonucleotides to form the insert were synthesized and obtained commercially (Genesis Labs, The Woodlands, Tex.), and the insert was generated by polymerase chain 45 reaction (PCR). The linked synthetic DNA encoding the mat.alpha. prepro/PEP-1 fusion protein was then incorporated by ligation into the modified pHIL-D2 plasmid between the BstBI and EcoRI sites.

The ligation products were used to transform Escherichia coli strain XL1 Blue. A PCR assay was used to screen E. coli transformants for the desired plasmid construct. DNA from cell extracts was amplified by PCR using primers containing the 5' AOX1 and 3' AOX1 sequences (see above and FIG. 2). PCR products of the correct number of base pairs were sequenced. In addition, approximately 20-50 bp on either side of the cloning sites were sequenced, and the predicted sequence was obtained. The final DNA sequence of the insert in the pHIL-D2 plasmid (to yield plasmid pPIC-K503) is shown in FIG. 2 along with portions of flanking 5' and 3' AOX1 sequences and corresponding amino acid sequence of the fusion protein comprising the mat.alpha. prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 KI polypeptide. A transformant with the desired expression plasmid construct, plasmid pPIC-K503, was selected for preparing yeast cell lines for routine production of PEP-1.

### Example 3

Manufacture of PEP-1 from Recombinant Yeast Cell Line Spheroplasts of P. pastoris GS115 having the His4.sup.- 5 phenotype were transformed with the expression plasmid

pPIC-K503 (above) following linearization of the plasmid at the SacI site and homologous recombination of the plasmid DNA into the host 5' AOX1 locus. The phenotype of the production strain is His4.sup.+. The entire plasmid was inserted into the 5' AOX1 genomic sequence of the yeast.

Isolates from the transformation were screened for growth in the absence of exogenous histidine with methanol as the sole carbon source. Greater than 95% of the transformants 15 retained the wild-type ability to grow with methanol as the sole carbon source, thereby demonstrating that the plasmid had been inserted into the host genome by homologous recombination rather than transplacement. These transformants did not require exogenous histidine for growth, thereby demonstrating that the plasmid had integrated into the host genome. Selected colonies were cloned. Small culture expression studies were performed to identify clones secreting the highest levels of active PEP-1 into the culture 25 medium. PEP-1 secretion levels in clarified culture supernatant solutions were quantified for PEP-1 levels by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and evaluated for kallikrein inhibition. A yeast clone was selected for PEP-I production based on its high level of 30 PEP-1 expression among cultures sampled.

Master and working cell banks of P. pastoris producing PBP-1 were prepared commercially (MDS Pharma Services, Bothell, Wash.). A standard production of PEP-1 in yeast 35 comprised three steps as follows: (1) preparation of the seed culture, (2) fermentation, and (3) recovery of the culture.

The seed culture step consisted of the inoculation of six flasks (300 mL each) containing sterile inoculum broth pH=5) with the contents of a single vial of a working cell bank of P. pastoris producing PEP-1. Flasks were inoculated in an orbital shaker (300 rpm) for approximately 13 hours at 30.degree. C.+-0.2.degree. C.

Fermentations were performed in a closed 100 liter Braun fermenter filled with sterile broth. Each fermentation was initiated with the transfer of the contents of the six seed culture flasks to the fermenter. After approximately 24 hours, the glycerol in the fermenter became exhausted and 50 additional glycerol was added for approximately 8 additional hours.

A mixed feed phase, which lasted approximately 83 hours, was then initiated by the addition of a glycerol and methanol feed. At the end of this time, the fermentation was 55 terminated, and the fermenter contents were diluted with purified water. The purification and processing of PEP-1 consisted of five steps as follows: (I) expanded bed chromatography, (2) cation exchange chromatography, (3) hydrophobic interaction chromatography (HIC), (4) ultrafiltration and diafiltration, and (5) final filtration and packaging.

The initial purification step consisted of expanded bed chromatography. The diluted fermenter culture was applied to the equilibrated column packed with Streamline SP resin (Amersham Pharmacia Streamline 200 chromatography col

umn, Amersham Pharmacia, Piscataway, N.J.). The column was then washed (50 mM acetic acid, pH=3.0-3.5) in an up-flow mode to flush the yeast cells from the expanded bed. The top adaptor was raised above the expanded bed enhance washing. The flow was stopped and the bed was allowed to settle. The adaptor was moved down so that it was slightly above the settled bed. The direction of the flow was reversed. The effluent was collected. Washing was continued in a downward mode using 50 mM sodium acetate, pH 4.0. The effluent was collected. PEP-1 was eluted from the column using 50 mM sodium acetate, pH 6.0. The chuate was collected in a 50 liter container. The cluate was then filtered through a 0.22.mu. filter into a clean container located in the purification site. Additional samples were collected for the determination of PEP-1 concentration. A cation exchange chromatography step was then performed using the filtered eluate from the expanded bed column. PEP-1 was eluted from the column using 15 mM trisodium citrate, pH 6.2.

Additional proteins were removed from the PEP-1 preparation by hydrophobic interaction chromatography (HIC). Prior to HIC, the eluate from the cation exchange column was diluted with ammonium sulfate. The cluate was applied to the column, and the PEP-1 was eluted using ammonium sulfate (0.572 M) in potassium phosphate (100 mM), pH 7.0. The cluate was collected in fractions based on A280 values. All fractions were collected into sterile, pre-weighed PETG bottles.

Selected fractions were pooled into a clean container. The pool was concentrated by ultrafiltration. The concentrated PEP-1 preparation was immediately diafiltered against ten volumes of PBS, pH 7.0.

A final filtration step was performed prior to packaging in order to minimize the bioburden in the bulk PEP-1. The bulksolution was filtered through a 0.22.mu. filter and collected into a sterile, pre-weighed PETG bottle. A sample was (yeast nitrogen base, potassium phosphate, and glycerol, 40 removed for lot release testing. The remainder of the bulk was dispensed aseptically into sterile PETG bottles and stored at -20.degree. C.

#### Example 4

Kallikrein Inhibition Assay

A kinetic test was used to measure inhibitory activity of KI polypeptides, such as PEP-1. The kinetic assay measures fluorescence following kallikrein-mediated cleavage of a substrate, prolylphenylalanylarginyl amino methyl coumarin. A known amount of kallikrein was incubated with a serially diluted KI polypeptide reference standard or serially diluted KI polypeptide test samples, in a suitable reaction buffer on a microtiter plate. Each sample was run in triplicate. The substrate solution was added, and the plate read immediately using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. At least two each of the reference standard and sample curves were required to have an R-squared value of 0.95 to be considered valid.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg Asn Asn Phe Lys Ser Ala 35 40 45
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<213> ORGANISM: Artificial Sequence

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    Net Thr Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Glu Thr
    Phe Gln Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu
    Lys Glu Cys Leu Gln Thr Cys Arg Thr Val
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   Thr Val Ala Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Arg Ala
1 5 10 15
  Phe Ile Gln Leu Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu
20 25 30
  Phe Pro Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu
  Lys Glu Cys Arg Glu Tyr Cys Gly Val Pro
50 55
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  <212> TYPE: PRT
  213> ORGANISM: Artificial Sequence
  <220> FEATURE:
  <223> OTHER INFORMATION: LACI-D1 Sequence
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 Ile Met Lys Arg Phe Phe Phe Asn Ile Phe Thr Arg Gin Cys Glu Glu
 Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu
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 <210> SEQ ID NO 33
 <211> LENGTH: 58
 <212> TYPE: PRT
213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LACI-D2 Sequence
<400> SEQUENCE: 33
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Tyr Ile Thr Arg Tyr Phe Tyr Asn Asn Gln Thr Lys Gln Cys Gln Arg
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Phe Lye Tyr Gly Gly Cys Leu Gly Asn Met Asn Asn Phe Glu Thr Leu

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   <211> LENGTH: 58
   <212> TYPE: PRT
   <213> ORGARISM: Artificial Sequence
   <220> FEATURE:
   <223> OTHER INFORMATION: LACI-D3 Sequence
   <400> SEQUENCE: 34
  Gly Pro Ser Trp Cys Leu Thr Pro Ala Asp Arg Gly Leu Cys Arg Ala
1 5 10 15
  Asn Glu Asn Arg Phe Tyr Tyr Asn Ser Val Ile Gly Lys Cys Arg Pro
  Phe Lys Tyr Ser Gly Cys Gly Gly Asn Glu Asn Asn Phe Thr Ser Lys
  Gln Glu Cys Leu Arg Ala Cys Lys Lys Gly
50 55
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  212> TYPE: PRT
  <213> ORGANISM: Artificial Sequence
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  <400> SEQUENCE: 35
 Leu Pro Asn Val Cys Ala Phe Pro Met Glu Lys Gly Pro Cys Gln Thr
1 5 10 15
 Tyr Met Thr Arg Trp Phe Phe Asn Phe Glu Thr Gly Glu Cys Glu Leu
20 25 30
 Phe Ala Tyr Gly Gly Cys Gly Gly Asm Ser Asm Asm Phe Leu Arg Lys
 Glu Lys Cys Glu Lys Phe Cys Lys Phe Thr
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Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Arg Asp
1 5 10 15
Phe Ile Leu Lys Trp Tyr Tyr Asp Pro Asn Thr Lys Ser Cys Ala Arg
20 25 30
Phe Trp Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly Ser Gln
35 40 45
Lys Glu Cys Glu Lys Val Cys Ala Pro Val
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<400> SEQUENCE: 37

Asn Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys Arg Ala 1 5 10 15

Len Leu Leu Arg Tyr Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys Arg Gln 20 25 30

Phe Leu Tyr Gly Gly Cys Glu Gly Asn Ale Asn Asn Phe Tyr Thr Trp

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<210> SEQ ID NO 38

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<213> ORGANISM: Artificial Sequence

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Val Pro Lys Val Cys Arg Leu Gln Val Ser Val Asp Asp Gln Cys Glu 1 5 10 15

Gly Ser Thr Glu Lys Tyr Phe Phe Asn Leu Ser Ser Met Thr Cys Glu

Lys Phe Phe Ser Gly Gly Cys His Arg Asn Arg Ile Glu Asn Arg Phe

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<210> SEQ ID NO 39

<211> LENGTH: 58

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> PEATURE:

<223> OTHER INFORMATION: TFPI-2 D3 Sequence

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Asn Val Thr Arg Tyr Tyr Phe Asn Pro Arg Tyr Arg Thr Cys Asp Ala 20 25 30

Phe Thr Tyr Thr Gly Cys Gly Gly Asn Asp Asn Asn Phe Val Ser Arg 35 40 45

Glu Asp Cys Lys Arg Ala Cys Ala Lys Ala 50 55

<210> SEQ ID NO 40

<211> LENGTH: 59

212> TYPE: PRT

213> ORGANISM: Artificial Sequence

<220> PERTURE:

<223> OTHER INFORMATION: APP-I Sequence

<400> SEQUENCE: 40

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Ala Met Ile Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala 20 25 30

Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr

Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala

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   <223> OTHER INFORMATION: EpiNE7 Sequence
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   Arg Pro Asp Phe Cys Lew Glu Pro Pro Tyr Thr Gly Pro Cys Val Ala
   Met Phe Pro Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Lea Cys Gln Thr
  Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Lys Ser Ala
  Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
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  <223> OTHER INFORMATION: BITI-E7-141 Sequence
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 Met Phe Pro Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Gln Thr
 Phe Val Tyr Gly Cly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu
 Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
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Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala
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Het Phe Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Gln Thr 20 25 30

Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu

Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala

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<211> LENGTH: 62

<212> TYPE: PRT

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<220> FEATURE:

<223> OTHER INFORMATION: EPI-HNE-1 Sequence

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Glu Ala Glu Ala Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly

Pro Cys Ile Ala Phe Phe Pro Arg Tyr Phe Tyr Asn Ala Lys Ala Gly
20 25 30

Leu Cys Gln Thr Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn

Phe Lys Ser Ala Glu Asp Cys Met Arg Thr Cys Gly Gly Ala 50 55 60

<210> SEQ ID NO 47

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<212> TYPE: PRT

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<220> FEATURE:

<223> OTHER INFORMATION: EPI-HNE-2 Sequence

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Pro Arg Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro

Tyr Gly Gly Cye Glu Gly Asn Cly Asn Lye Phe Tyr Ser Glu Lye Glu

Cys Arg Glu Tyr Cys Gly Val Pro

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      Pro Arg Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu Phe Pro
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    Cys Arg Glu Tyr Cys Gly Val Pro
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  Glu Ala Val Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys
  Ile Ala Phe Phe Pro Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys
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Arg Gln Phe Len Tyr Gly Gly Cys Glu Gly Asu Ala Asn Asn Phe Tyr

35 40 45 Thr Trp Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile 55 60 <210> SEQ ID NO 52 <211> LENGTH: 60 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> PEATURE: <223> OTHER INFORMATION: DPI68 KR Sequence <400> SEQUENCE: 52 Glu Ala Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Pro Gly Ile Cys Ile Gly Phe Phe Pro Arg Tyr Phe Tyr Asn Asn Gln Ala Lys Gln Cys 25 30 Glu Arg Phe Val Tyr Gly Gly Cys Leu Gly Asn Met Asn Asn Phe Glu Thr Leu Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly 55 <210> SEQ ID NO 53 <211> LENGTH: 60 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: DPI84 KR Sequence <400> SEQUENCE: 53 Glu Ala Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Ile Ala Phe Phe Pro Arg Trp Tyr Tyr Asp Pro Asn Thr Lys Ser Cys Ala Arg Phe Val Tyr Gly Gly Cye Gly Asn Glu Asn Lye Phe Gly Ser Gln Lys Glu Cys Glu Lys Val Cys Ala Pro Val <210> SEQ ID NO 54 211> LENGTH: 58 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: VARIANT <220> FEATURE: <221> NAME/KEY: VARIANT <222> LOCATION: 10 <223> OTHER INFORMATION: Kaa = Asp or Glu <220> FEATURE: <221> NAME/KEY: VARIANT <222> LOCATION: 11 <223> OTHER INFORMATION: Kea = Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr <221> NAME/KEY: VARIANT <222> LOCATION: 13 <223> OTHER INFORMATION: Xaa = Arg, His, Pro, Asa, Ser, Thr, Ala, Gly, <220> PEATURE: <221> NAME/KEY: VARIANT <222> LOCATION: 15 <223> OTHER INFORMATION: Kaa = Arg, Ala, Ser, Gly, Met, Asn or Gln <221> Name/Kry: Variant <222> LOCATION: 16 <223> OTHER INFORMATION: Xaa = Ala, Gly, Ser, Asp or Asn <221> NAME/KEY: VARIANT

34 (4)

#### -continued

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  <222> LOCATION: 18
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 <220> PEATURE:
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 <220> FEATURE:
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Met His Ser Phe Cys Ala Phe Lys Ala Xaa Xaa Gly Xaa Cys Xaa Xaa
Xaa Xaa Xaa Arg Xaa Phe Phe Asn Ile Phe Thr Arg Gln Cys Xaa Xaa
Phe Kaa Kaa Gly Gly Cys Kaa Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
    50
```

#### What is claimed is:

1. An isolated polypeptide comprising the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein.

2. The isolated polypeptide of claim 1, wherein the polypeptide comprises the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).

3. The isolated polypeptide of claim 1, wherein the polypeptide consists of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2).

4. The isolated polypeptide of claim 2, wherein the polypeptide consists of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp (SEQ ID NO:2).

In re U.S. Patent No.: 7,276,48

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

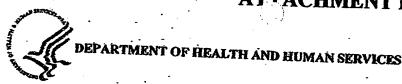
Attorney Docket No.: D2033-

094003

Attachment D

Biologics License Approval Letter including enclosures

### ATTACHMENT D



**Food and Drug Administration**Silver Spring MD 20993

Our STN: BL 125277/0

BLA APPROVAL December 1, 2009

Dyax Corporation 300 Technology Square Cambridge, MA 02139

Attention:

Nicole D'Auteuil

Senior Director, Regulatory Affairs

Dear Ms. D'Auteuil:

Please refer to your biologics license application (BLA), dated September 23, 2008, received September 23, 2008, submitted under section 351 of the Public Health Service Act (PHSA) for Kalbitor (ecallantide) injection.

We acknowledge receipt of your submissions dated December 31, 2007, March 27, September 23, October 10 and 30, November 13, 18, and 26, and December 9, 11, 15, 19 (2), 23, 24, and 31, 2008, and January 5, 9, 13, 16, 21, 23, 27, 28, and 29, February 11, 12, 13, 20, and 27, March 9, May 31, June 10 and 29, July 21, August 12 and 31, September 29, October 26 (3) and 30 (2), November 9, 16, 17, 18, 19, 23 (2), 24, 25, and 27, and December 1, 2009.

The May 31, 2009, submission constituted a complete response to our March 25, 2009, action letter.

We have completed our review of your application and are issuing Department of Health and Human Services U.S. License No. 1789 to Dyax Corporation, Cambridge, Massachusetts, under the provisions of section 351(a) of the PHSA controlling the manufacture and sale of biological products. The license authorizes you to introduce into, or deliver for introduction into, interstate commerce those products for which your company has demonstrated compliance with establishment and product standards.

Under this license, you are authorized to manufacture the product Kalbitor (ecallantide) injection. Kalbitor (ecallantide) injection is indicated for the treatment of acute attacks of hereditary angioedema in patients 16 years of age and older.

Under this license, you are approved to manufacture ecallantide drug substance at Avecia Biologics in Billingham, United Kingdom. The final formulated product will be manufactured, filled, labeled, and packaged at Hollister-Stier Laboratories, LLC, Spokane, Washington. You may label your product with the proprietary name Kalbitor and market it as a sterile liquid in single-dose, 2-mL glass vials (1-mL fill), 10 mg/mL for subcutaneous injection.

You currently are not required to submit samples of future lots of Kalbitor (ecallantide) injection to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging, or labeling of Kalbitor (ecallantide) injection, or in the manufacturing facilities.

#### REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

Because this biological product for this indication has an orphan drug designation, you are exempt from this requirement.

#### POSTMARKETING REQUIREMENTS UNDER 505(6)

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a known serious risk of hypersensitivity reactions and immunogenicity, a theoretical risk of disordered coagulation, and an unexpected, serious risk of malignancy with use of Kalbitor (ecallantide) injection.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess these serious risks.

Therefore, based on appropriate scientific data, FDA has determined that you are required to conduct the following:

1. A long-term, observational safety study with Kalbitor (ecallantide) in patients with hereditary angioedema to evaluate hypersensitivity, immunogenicity, and coagulation disorders. The study should include the following objectives: 1) identify predictive risk factors and develop effective screening tools to mitigate the risk of hypersensitivity and anaphylaxis; 2) correlate antibody levels with adverse events and lack of efficacy; and 3) evaluate the risk of hypercoagulability and hypocoagulability.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Protocol Submission:

December 2009

**Study Completion Date:** 

February 2014

**Final Report Submission:** 

August 2014

2. Establish the sensitivity and cutpoint for the anti-ecallantide neutralizing antibody assay, using immunoaffinity-purified ecallantide-specific human IgG.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Report Submission:

March 2010

Evaluate for cross-reactivity of anti-ecallantide antibodies with TFPI, perform studies to
determine if human anti-ecallantide antibodies bind TFPI, and perform suitability studies
and epitope mapping of the human anti-ecallantide antibody response if binding is
observed.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

**Final Report Submission:** 

August 2010

4. Develop and validate anti-ecallantide and anti-P. pastoris-specific human IgE detection assays using a sensitive platform such as ECL. Such assays should be free from interference by anti-ecallantide IgG antibodies.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Method Development Reports Submission:

April 2010

Final Report Submission:

September 2010

 A study in rats to evaluate the carcinogenic potential of Kalbitor (ecallantide). The sixmonth subcutaneous toxicology study with rats could serve as the basis of dose selection.

The timetable you submitted on November 19, 2009, states that you will conduct this study according to the following timetable:

Final Protocol Submission:

June 2010

**Study Completion Date:** 

September 2012

Final Report Submission:

September 2013

Submit the protocols to your IND, with a cross-reference letter to this BLA 125277. Submit all final reports to your BLA 125277. Prominently identify submissions with the following wording in bold capital letters at the top of the first page of the submission, as appropriate:

- REQUIRED POSTMARKETING PROTOCOL UNDER 505(0)
- REQUIRED POSTMARKETING FINAL REPORT UNDER 505(0)
- REQUIRED POSTMARKETING CORRESPONDENCE UNDER 505(0)

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

# POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS OF SECTION 506B

We acknowledge your written commitments as described in your letter of November 24, 2009, as outlined below:

6. The submission, as a pre-approval supplement, of an updated stability protocol for drug product that will add an accelerated or stress stability condition as part of the annual stability program. The data accumulated from this protocol will be submitted to the BLA on an annual basis.

Final Protocol Submission: January 2010

7. To evaluate the minimum fill volume required to provide appropriate dosage withdrawal and whether an adjustment to the fill volume for the drug product is necessary to reduce the likelihood that a patient will be overdosed with any excess drug product. The final study report including identification of a new fill volume, if found to be necessary, will be provided. Should the fill volume need to be changed, this report will include a proposed execution plan.

Final Report Submission: April 2010

# RISK EVALUATION AND MITIGATION STRATEGY REQUIREMENTS

Section 505-1 of the FDCA authorizes FDA to require the submission of a Risk Evaluation and Mitigation Strategy (REMS) if FDA determines that such a strategy is necessary to ensure that the benefits of the drug outweigh the risks (section 505-1(a)).

Your proposed REMS, submitted on December 1, 2009, and appended to this letter, is approved. The REMS consists of a Medication Guide, a communication plan, and a timetable for submission of assessments of the REMS.

The REMS assessment plan should include but is not limited to the following:

- a. A summary of all reported serious hypersensitivity reactions with analysis of adverse event reporting by prescriber type.
- Specification of measures that would be taken to increase awareness if surveys of health care providers indicate that provider awareness is not adequate.
- c. An evaluation of health care providers' understanding and patients' understanding of the serious risks of Kalbitor (ecallantide) injection.
- d. Based on the information submitted, an assessment and conclusion of whether the REMS is meeting its goals, and whether modifications to the REMS are needed.

Assessments of an approved REMS must also include, under section 505-1(g)(3)(B) and (C), information on the status of any post-approval study or clinical trial required under section 505(o) or otherwise undertaken to investigate a safety issue. You can satisfy these requirements in your REMS assessments by referring to relevant information included in the most recent annual report required under section 506B, and 21 CFR 601.70, and including any updates to the status information since the annual report was prepared. Failure to comply with the REMS assessments provisions in section 505-1(g) could result in enforcement action.

We remind you that in addition to the assessments submitted according to the timetable included in the approved REMS, you must submit a REMS assessment and may propose a modification to the approved REMS when you submit a supplemental application for a new indication for use as described in section 505-1(g)(2)(A) of FDCA.

Prominently identify the submission containing the REMS assessments or proposed modifications with the following wording in bold capital letters at the top of the first page of the submission:

BLA 125277 REMS ASSESSMENT NEW SUPPLEMENT FOR BLA 125277 PROPOSED REMS MODIFICATION REMS ASSESSMENT

NEW SUPPLEMENT (NEW INDICATION FOR USE) FOR BLA 125277 REMS ASSESSMENT PROPOSED REMS MODIFICATION (if included)

If you do not submit electronically, please send five copies of REMS-related submissions.

We request that the labeling approved today be available on your website within 10 days of product launch.

### REPORTING REQUIREMENTS

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). In addition, you should submit all reports of serious anaphylactic or hypersensitivity events within 15 days of receipt as 15-day expedited reports. You should submit postmarketing adverse experience reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266.

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at <a href="http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm">http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm</a>.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA 3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations sent by courier or overnight mail should be addressed to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
10903 New Hampshire Avenue, Bldg. 51, Room 4203
Silver Spring, MD 20992-0002

### CONTENT OF LABELING

Within 14 days of the date of this letter, submit content of labeling (21 CFR 601.14(b)) in structured product labeling (SPL) format, as described at <a href="http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm">http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm</a> that is identical in content to the enclosed labeling (text for the package insert and Medication Guide submitted November 27, 2009). The content of labeling should be submitted by updating your application by referencing the SPL file submitted to the drug establishment registration and drug listing system. To do this, place a link in your application submission that directs FDA to your SPL file. For administrative purposes, please designate this submission "Product Correspondence - Final SPL for approved BLA STN 125277." In addition, within 14 days of the date of this letter, amend any pending supplements for this BLA with content of labeling in SPL format to include the changes approved in this supplement. For additional information on submitting labeling to drug establishment registration and drug listing and to applications, see the FDA guidances at

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072339.pdf and

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf.

# CARTON AND CONTAINER LABELS

Submit final printed carton and container labels that are identical to the labels submitted November 23, 2009, as soon as they are available but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005). Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "Product Correspondence – Final Printed Carton and Container Labels for approved BLA STN 125277." Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with labeling that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

# PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

You must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications, see <a href="http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm">http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm</a>.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

# LETTERS TO HEALTH CARE PROFESSIONALS

If you issue a letter communicating important safety-related information about this drug product (i.e., a "Dear Health Care Professional" letter), we request that you submit an electronic copy of the letter to both this BLA and to the following address:

MedWatch Food and Drug Administration Suite 12B-05 5600 Fishers Lane Rockville, MD 20857

# POST-ACTION FEEDBACK MEETING

New molecular entities and important new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during the drug development and marketing application review process. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, contact the Division of Pulmonary and Allergy Products.

If you have any questions, contact the Senior Regulatory Health Project Manager, Colette Jackson, at (301) 796-1230.

Sincerely,

/Curtis J. Rosebraugh, M.D., M.P.H./

Curtis J. Rosebraugh, M.D., M.P.H.

Director

Office of Drug Evaluation II

Center for Drug Evaluation and Research

Enclosures:

REMS documents
Package Insert
Medication Guide

Carton and Container Labels

3. Distribution of materials: Communication plan materials will be distributed at the same time as product launch.

Direct Mail: Dyax will issue the DHCP Letter to targeted healthcare providers at the time of product launch and yearly for 2 years thereafter. In addition, for 2 years after launch, any known new prescribers of KALBITOR not previously targeted will also be sent the DHCP Letter. The DHCP Letter will include the warnings associated with KALBITOR and will describe symptoms of anaphylaxis that may overlap with presenting symptoms of an attack of HAE. The DHCP Letter will be sent by direct mail to providers in the specialties of Allergy/Immunology and Emergency Medicine. The DHCP Letter will include the Full Prescribing Information and the Medication Guide. Copies of these materials will also be available through a stand-alone webpage accessed through the product web site. (see attached webpage)

Dyax Representatives: The DHCP Letter will be provided with the Full Prescribing Information and Medication Guide by Dyax sales representatives to potential prescribers during the first discussion of KALBITOR during the first year of product availability.

The communication material listed in Section B.2 above will also be available at the time of approval by calling Dyax at 1-888-452-5248.

## C. Elements to Assure Safe Use

Elements to Assure Safe Use are not required.

# D. Implementation System

An Implementation System is not required.

# E. Timetable for Submission of Assessments

REMS assessments will be submitted at 18 months, 3 years and 7 years after approval. The reporting interval covered by each assessment will conclude no earlier than 60 days before the submission date for that assessment time interval. Each assessment will be submitted so that it is received by the FDA on or before the due date.

### [on Dyax letterhead]

### IMPORTANT DRUG WARNING

### Dear Healthcare Professional:

Dyax Corp. is writing to inform you of important safety information for KALBITOR® (ecallantide). KALBITOR is a subcutaneous injection indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age or older. Important safety information related to KALBITOR includes:

- The risk of anaphylaxis
- The need to distinguish signs and symptoms of anaphylaxis from HAE attacks

To ensure that the benefits of KALBITOR treatment outweigh the risks, the KALBITOR labeling includes a boxed warning concerning anaphylaxis, as follows:

WARNING: Anaphylaxis

Anaphylaxis has been reported after administration of KALBITOR<sup>®</sup>. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR.

In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical trials, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. In clinical trials, when hypersensitivity was observed, it usually occurred immediately following exposure to KALBITOR, and always within the first hour following dosing.

In order to appropriately manage anaphylaxis, it must be recognized if it occurs. Because the signs and symptoms of HAE attacks may overlap with the signs and symptoms of anaphylaxis, there is a need to distinguish between serious hypersensitivity, including anaphylaxis and HAE attack symptoms.

Signs and symptoms that can be seen in either anaphylaxis or acute attacks of HAE include:

- erythema of the skin
- laryngeal edema
- dyspnea
- flushing
- stomach and gastrointestinal symptoms
- decreases in blood pressure

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. If a patient does not respond to an initial dose of KALBITOR, an additional dose of KALBITOR may be administered within a 24 hour period; before administering a repeat dose of KALBITOR, it is very important to assess the patient to assure that symptoms are reflective of an HAE attack and not a hypersensitivity reaction.

Please take time to read the enclosed KALBITOR Package Insert for full prescribing information.

In addition, please review the attached Medication Guide with each patient who is prescribed KALBITOR. The Medication Guide must be given to patients upon each use of KALBITOR and is supplied with each dose unit.

To report adverse events potentially associated with KALBITOR, please call Dyax Corp. at 1-888-452-5248. Alternatively, adverse event information may be reported to FDA's MedWatch Reporting System by:

- o Phone at I-800-FDA-1088 (1-800-332-1088)
- o Facsimile at 1-800-FDA-0178 (1-800-332-0178)
- o Mail using FDA Form 3500 located at http://www.fda.gov/medwatch

We invite you to contact Dyax Corp. at 1-888-452-5248 if you have any questions about KALBITOR or the information in this letter.

Sincerely,

Patrick T. Horn, MD, PhD Vice President, Clinical and Medical Affairs Dyax Corp.

Enclosures: Full Prescribing Information and Medication Guide





# IMPORTANT SAFETY INFORMATION FOR HEALTHCARE PROFESSIONALS

### Risk Evaluation and Mitigation Strategy (REMS)

A Risk Evaluation and Mitigation Strategy (REMS) is a strategy to manage known or potential serious risks associated with a drug product and is required by the Food and Drug Administration to ensure that the benefits of the drug outweigh its risks.

In order for Dyax to communicate certain risks to ensure that KALBITOR is prescribed and taken safely, Dyax has worked with the FDA to develop materials to communicate the risk of anaphylaxis and the importance of distinguishing between hypersensitivity reactions and ongoing hereditary angroedema (HAE) syroptoms. The REMS program is designed to inform healthcare providers and patients about the potential risks with KALBITOR. To learn more about serious risks, read the important safety information provided in this link, including the Medication Guide.

#### The goals of the KALBITOR REMS are:

- To inform healthcare providers about the risk of anaphylaxis associated with KALSITOR and the importance of distinguishing between a hypersensitivity reaction and hereditary angloederna (HAE) attack symptoms.
- To educate patients about the serious risks associated with KALBITOR therapy.

To download the REMS documents:

-: Dear Healthcare Professional Letter odf

Prescribing Information.pdf

Medication Guide, adf

#### mportant Safety Information

IARNING: Anaphylaxis

naphylaxis has been reported after administration of KALBITOR®. Becouse of the risk of anaphylaxis, KALBITOR should only be administered by a realthcare professional ith appropriate medical support to manage anaphylexis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between persensitivity reactions and hereditary anginedema and patients strough be manufored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity

#### INTRAINDICATIONS

) not administer KALDITOR to a padent who has known clinical hypersensitivity to KALBITOR

#### ARNINGS AND PRECAUTIONS

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lents should be observed for an appropriate period of time after administration of KALBITOR, raking into excount the time to onset of anaphytaxis seen in clinical trials. en the similarity in trypersensionity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

#### **VERSE EUFNIS**

most common adverse events (23% and greater than placebo) in HAE patients were headache, hauses, diarries, pyrexia. Injection site reactions, and hasopharyneids. re is a potential for enmunogenicity with the use of KALBITOR. Patients who serotorivers may be at a higher risk of a hypersensitivity reaction. The long-term effects of

Compared in the ECTER in parameter thank the course of agent can had become expendenced

se see the विदेशियका भूतक भूतिभाग्रहात्व including Boxed Warning and Medication Guide.

thcare professionals should report all suspected adverse events associated with the use of KALBITOR. Please contact Dyax Corp. at 1-888-452-5248. Alternatively, this mation may be reported to the FDA MedWatch System by phone at 1-800-FDA-1088 or by mail using Form 3500 at www.fda.gov/medwatch.

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Dyax Corp.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use
KALBITOR® safely and effectively. See full prescribing information for
KALBITOR.

KALBITOR (ecollantide) injection, for subcutaneous use initial U.S. Approval: [year]

WARNING -Ausphylaxis
(See full prescribing information for complete boxed warning)

Anaphylaxis has been reponted after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4), Warnings and Precontions (5.1), and Adverse Reactions (6)].

- KALBITOR is a plasma kallikrein inhibitor indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older. (1)
- DOSAGE AND ADMINISTRATION

  30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If an attack persists, an additional dose of 30 mg may be administered within a 24 hour period. (2.1)

  KALBITOR should only be administered by a healthcare
  - KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema. (2.2).

DOSAGE FORMS AND STRENGTHS

Single use glass vial containing 10 mg/ml. of ecallartide as a solution for injection. (3)

68153c

-----CONTRAINDICATIONS

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. (4)

-- WARNINGS AND PRECAUTIONS--

- Hypersensitivity Reactions Including Anaphylanis: Anaphylanis has
  occurred in 3.9% of treated patients. Administer KALBITOR in a
  setting equipped to manage anaphylanis and hereditary angioedema.
  Given the similarity in hypersensitivity symptoms and acute HAE
  symptoms, monitor patients closely for hypersensitivity reactions (5).
- The most common adverse reactions occurring in ≥3% of KALBITORtreated patients and greater than placebo are headache, nausea, diarrhea, pyrexia, injection site reactions, and nasopharyngitis. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Dyex Corp. at 1-888-452-5248 or FDA at 1-800-FDA-1088 or www.fda.gow/medwatch

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide

Revised: [m/year]

#### Full prescribing information: contents\* Warning: anaphylaxis

- I INDICATIONS AND USAGE
- DOSAGE AND ADMINISTRATION
  - 2.1 Recommended Dosing
    - 2.2 Administration Instructions
- 3 DOSAGE FORMS AND STRENGTHS
- 4 CONTRAINDICATIONS
- 5 WARNINGS AND PRECAUTIONS
  - 5.1 Hypersensitivity Reactions, Including Anaphylaxis
- 6 ADVERSE REACTIONS
  - 6.1 Chinical Trials Experience
- 6.2 Immunogenicity
- 7 DRUG INTERACTIONS
- USE IN SPECIFIC POPULATIONS
  - 8.1 Pregnancy
  - 8.2 Labor and Delivery
  - 8.3 Nursing Mothers
  - 8.4 Pediatric Use
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- 18 OVERDOSAGE
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  - 12.1 Mechanism of Action
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- 13 NONCLINICAL TOXICOLOGY
  - 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility
  - 13.2 Animal Toxicology
- 14 CLINICAL STUDIES
- 16 HOW SUPPLIED/STORAGE AND HANDLING
- 17 PATIENT COUNSELING INFORMATION

<sup>\*</sup>Sections or subsections omitted from the full prescribing information are not listed.

# **FULL PRESCRIBING INFORMATION**

### WARNING: Anaphylaxis

Anaphylaxis has been reported after administration of KALBITOR. Because of the risk of anaphylaxis, KALBITOR should only be administered by a healthcare professional with appropriate inedical support to manage anaphylaxis and hereditary angioedema. Healthcare professionals should be aware of the similarity of symptoms between hypersensitivity reactions and hereditary angioedema and patients should be monitored closely. Do not administer KALBITOR to patients with known clinical hypersensitivity to KALBITOR. [see Contraindications (4), Warnings and Precautions (5.1), and Adverse Reactions (6)]

# 1 INDICATIONS AND USAGE

KALBITOR® (ecallantide) is indicated for treatment of acute attacks of hereditary angioedema (HAE) in patients 16 years of age and older.

# 2 DOSAGE AND ADMINISTRATION

### 2.1 Recommended Dosing

The recommended dose of KALBITOR is 30 mg (3 mL), administered subcutaneously in three 10 mg (1 mL) injections. If the attack persists, an additional dose of 30 mg may be administered within a 24 hour period.

### 2.2 Administration Instructions

KALBITOR should only be administered by a healthcare professional with appropriate medical support to manage anaphylaxis and hereditary angioedema.

KALBITOR should be refrigerated and protected from the light. KALBITOR is a clear, colorless liquid; visually inspect each vial for particulate matter and discoloration prior to administration. If there is particulate matter or discoloration, the vial should not be used.

Using aseptic technique, withdraw 1 mL (10 mg) of KALBITOR from the vial using a large bore needle. Change the needle on the syringe to a needle suitable for subcutaneous injection. The recommended needle size is 27 gauge. Inject KALBITOR into the skin of the abdomen, thigh, or upper arm. Repeat the procedure for each of the 3 vials comprising the KALBITOR dose. The injection site for each of the injections may be in the same or in different anatomic locations (abdomen, thigh, upper arm). There is no need for site rotation. Injection sites should be separated by at least 2 inches (5 cm) and away from the anatomical site of attack.

The same instructions apply to an additional dose administered within 24 hours. Different injection sites or the same anatomical location (as used for the first administration) may be used.

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Dyax Corp.

Dyax Corp.

# 3 DOSAGE FORMS AND STRENGTHS

KALBITOR is a clear, colorless liquid free of preservatives. Each vial of KALBITOR contains ecallantide at a concentration of 10 mg/mL.

### 4 CONTRAINDICATIONS

Do not administer KALBITOR to a patient who has known clinical hypersensitivity to KALBITOR. [see Warnings and Precautions (5.1)].

# 5 WARNINGS AND PRECAUTIONS

# 5.1 Hypersensitivity Reactions, Including Anaphylaxis

Potentially serious hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR. In 255 HAE patients treated with intravenous or subcutaneous KALBITOR in clinical studies, 10 patients (3.9%) experienced anaphylaxis. For the subgroup of 187 patients treated with subcutaneous KALBITOR, 5 patients (2.7%) experienced anaphylaxis. Symptoms associated with these reactions have included chest discomfort, flushing, pharyngeal edema, pruritus, rhinorrhea, sneezing, nasal congestion, throat irritation, urticaria, wheezing, and hypotension. These reactions occurred within the first hour after dosing.

Other adverse reactions indicative of hypersensitivity reactions included the following: pruritus (5.1%), rash (3.1%), and urticaria (2.0%).

Patients should be observed for an appropriate period of time after administration of KALBITOR, taking into account the time to onset of anaphylaxis seen in clinical trials. Given the similarity in hypersensitivity symptoms and acute HAE symptoms, patients should be monitored closely in the event of a hypersensitivity reaction.

KALBITOR should not be administered to any patients with known clinical hypersensitivity to KALBITOR [see Contraindications (4)].

### 6 ADVERSE REACTIONS

Hypersensitivity reactions, including anaphylaxis, have occurred in patients treated with KALBITOR [see Contraindications (4) and Warnings and Precautions (5.1)].

### 6.1 Clinical Trials Experience

Because clinical trials are conducted under varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The safety data described below reflect exposure to KALBITOR in 255 patients with HAE treated with either intravenous or subcutaneous KALBITOR. Of the 255 patients,

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66% of patients were female and 86% were Caucasian. Patients treated with KALBITOR were between the ages of 10 and 78 years.

Overall, the most common adverse reactions in 255 patients with HAE were headache (16.1%), nausea (12.9%), fatigue (11.8%), diarrhea (10.6%), upper respiratory tract infection (8.2%), injection site reactions (7.4%), nasopharyngitis (5.9%), vomiting (5.5%), pruritus (5.1%), upper abdominal pain (5.1%), and pyrexia (4.7%). Anaphylaxis was reported in 3.9% of patients with HAE: Injection site reactions were characterized by local pruritus, erythema, pain, irritation, urticaria, and/or bruising.

The incidence of adverse reactions below is based upon 2 placebo-controlled, clinical trials (EDEMA3® and EDEMA4®) in a total of 143 unique patients with HAE. Patients were treated with KALBITOR 30 mg subcutaneous or placebo. Patients were permitted to participate sequentially in both placebo-controlled trials; safety data collected during exposure to KALBITOR was attributed to treatment with KALBITOR, and safety data collected during exposure to placebo was attributed to treatment with placebo. Table 1 shows adverse reactions occurring in ≥3% of KALBITOR-treated patients that also occurred at a higher rate than in the placebo-treated patients in the two controlled trials (EDEMA3 and EDEMA4) of the 30 mg subcutaneous dose.

Table 1: Adverse Reactions Occurring at ≥3% and Higher than Placebo in 2 Placebo Controlled Clinical Trials in Patients with HAE Treated with KALBITOR

Adverse Reactions	Inical Trials in Patients with HAE  KALBITOR  N=100	Placetio N=81
Realtions	n (%) <sup>e</sup>	n (%)*
Headache	8 (8%)	
Nausea	5 (5%)	6 (7%)
Diamhea		i (1%)
Pyrexia	4 (4%)	3 (4%)
njection site reactions	4 (4%)	0
Nasopharyngitis	3 (3%)	1 (1%)
Patients experiencing more than	3 (3%)	o ´

Patients experiencing more than I event with the same preferred term are counted only once for that preferred term.

Some patients in EDEMA3 and EDEMA4 received a second, open-label 30 mg subcutaneous dose of KALBITOR within 24 hours following the initial dose. Adverse reactions reported by these patients who received the additional 30 mg subcutaneous dose of KALBITOR were consistent with those reported in the patients receiving a single dose.

## 6.2 Immunogenicity

In the KALBITOR HAE program, patients developed antibodies to KALBITOR. Rates of seroconversion increased with exposure to KALBITOR over time. Overall, 7.4% of patients seroconverted to anti-ecallantide antibodies. Neutralizing antibodies to ecallantide were determined in vitro to be present in 4.7% of patients.

Anti-ecallantide and anti-P. pastoris IgE antibodies were also detected. Patients who seroconvert may be at a higher risk of a hypersensitivity reaction. The long-term effects of antibodies to KALBITOR are not known.

The test results for the ecallantide program were determined using one of two assay formats: ELISA and bridging electrochemiluminescence (ECL). As with all therapeutic proteins, there is a potential for immunogenicity with the use of KALBITOR. The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to KALBITOR with the incidence of antibodies to other products may be misleading.

### 7 DRUG INTERACTIONS

No formal drug interactions studies were performed. No in vitro metabolism studies were performed.

### 8 USE IN SPECIFIC POPULATIONS

#### 8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled trials of KALBITOR in pregnant women. KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Because animal reproductive studies are not always predictive of human response, KALBITOR should be used during pregnancy only if clearly needed.

In rats, intravenous KALBITOR at an intravenous dose approximately 13 times the maximum recommended human dose (MRHD) on a mg/kg basis caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. No development toxicity was observed in rats that received an intravenous dose approximately 8 times the MRHD on a mg/kg basis. There were no adverse effects of KALBITOR on embryofetal development in rats that received subcutaneous doses up to approximately 2.4 times the MRHD on an AUC basis, and in rabbits that received intravenous doses up to approximately 6 times the MRHD on an AUC basis.

### 8.2 Labor and Delivery

No information is available on the effects of KALBITOR during labor and delivery.

### 8.3 Nursing Mothers

It is not known whether ecallantide is excreted in human milk. Caution should be exercised when ecallantide is administered to a nursing woman.

#### 8.4 Pediatric Use

Safety and effectiveness of KALBITOR in patients below 16 years of age have not been established.

#### 8.5 Geriatric Use

Clinical trials of KALBITOR did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

#### 10 OVERDOSAGE

There have been no reports of overdose with KALBITOR. HAE patients have received single doses up to 90 mg intravenously without evidence of dose-related toxicity. No deaths occurred in monkeys that received intravenous or subcutaneous doses up to 25 mg/kg (approximately 22 times the MRHD on an AUC basis).

#### 11 DESCRIPTION

KALBITOR (ecallantide) is a human plasma kallikrein inhibitor for injection for subcutaneous use.

KALBITOR is a clear and colorless, sterile, and nonpyrogenic solution. Each vial contains 10 mg ecallantide as the active ingredient, and the following inactive ingredients: 0.76 mg disodium hydrogen orthophosphate (dihydrate), 0.2 mg monopotassium phosphate, 0.2 mg potassium chloride, and 8 mg sodium chloride in water for injection, USP. KALBITOR is preservative free, with a pH of approximately 7.0. A 30 mg dose is supplied as 3 vials each containing 1 mL of 10 mg/mL KALBITOR. Each vial contains a slight overfill. Vials are intended for single use. Ecallantide is a 60-amino-acid protein produced in *Pichia pastoris* yeast cells by recombinant DNA technology.

### 12 CLINICAL PHARMACOLOGY

#### 12.1 Mechanism of Action

Hereditary angioedema (HAE) is a rare genetic disorder caused by mutations to C1-esterase-inhibitor (C1-INH) located on Chromosome 11q and inherited as an autosomal dominant trait. HAE is characterized by low levels of C1-INH activity and low levels of C4. C1-INH functions to regulate the activation of the complement and intrinsic coagulation (contact system pathway) and is a major endogenous inhibitor of plasma kallikrein. The kallikrein-kinin system is a complex proteolytic cascade involved in the initiation of both inflammatory and coagulation pathways. One critical aspect of this pathway is the conversion of High Molecular Weight (HMW) kininogen to bradykinin by the protease plasma kallikrein. In HAE, normal regulation of plasma kallikrein activity and the classical complement cascade is therefore not present. During

attacks, unregulated activity of plasma kallikrein results in excessive bradykinin generation. Bradykinin is a vasodilator which is thought by some to be responsible for the characteristic HAE symptoms of localized swelling, inflammation, and pain.

KALBITOR is a potent (Ki = 25 pM), selective, reversible inhibitor of plasma kallikrein. KALBITOR binds to plasma kallikrein and blocks its binding site, inhibiting the conversion of HMW kininogen to bradykinin. By directly inhibiting plasma kallikrein, KALBITOR reduces the conversion of HMW kininogen to bradykinin and thereby treats symptoms of the disease during acute episodic attacks of HAE.

#### 12.2 Pharmacodynamics

No exposure-response relationships for KALBITOR to components of the complement or kallikrein-kinin pathways have been established.

The effect of KALBITOR on activated partial thromboplastin time (aPTT) was measured because of potential effect on the intrinsic coagulation pathway. Prolongation of aPTT has been observed following intravenous dosing of KALBITOR at doses ≥20 mg/m². At 80 mg administered intravenously in healthy subjects, aPTT values were prolonged approximately two-fold over baseline values and returned to normal by 4 hours post-dose.

For patients taking KALBITOR, no significant QT prolongation has been seen. In a randomized, placebo-controlled trial (EDEMA4) studying the 30 mg subcutaneous dose versus placebo, 12-lead ECGs were obtained at baseline, 2 hours and 4 hours post-dose (covering the time of expected  $C_{max}$ ), and at follow-up (day 7). ECGs were evaluated for PR interval, QRS complex, and QTc interval. KALBITOR had no significant effect on the QTc interval, heart rate, or any other components of the ECG.

#### 12.3 Pharmacokinetics

Following the administration of a single 30 mg subcutaneous dose of KALBITOR to healthy subjects, a mean ( $\pm$  standard deviation) maximum plasma concentration of  $586 \pm 106$  ng/mL was observed approximately 2 to 3 hours post-dose. The mean area under the concentration-time curve was  $3017 \pm 402$  ng\*hr/mL. Following administration, plasma concentration declined with a mean elimination half-life of  $2.0 \pm 0.5$  hours. Plasma clearance was  $153 \pm 20$  mL/min and the volume of distribution was  $26.4 \pm 7.8$  L. Based on a population pharmacokinetic analysis, body weight, age, and gender were not found to affect KALBITOR exposure significantly. Ecallantide is a small protein (7054 Da) and renal elimination in the urine of treated subjects has been demonstrated.

No pharmacokinetic data are available in patients or subjects with hepatic or renal impairment.

# 13 NONCLINICAL TOXICOLOGY

# 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There are no animal or human studies to assess the carcinogenic or mutagenic potential of KALBITOR (ecallantide).

KALBITOR had no effects on fertility and reproductive performance in rats at subcutaneous doses up to 25 mg/kg/day (approximately 21 times the MRHD on a mg/kg basis).

### 13.2 Animal Toxicology

Reproductive Toxicology Studies

KALBITOR has been shown to cause developmental toxicity in rats, but not rabbits. Treatment of rats with an intravenous dose of 15 mg/kg/day (approximately 13 times the MRHD on a mg/kg basis) caused increased numbers of early resorptions and percentages of resorbed conceptuses per litter in the presence of mild maternal toxicity. However, no development toxicity was observed in rats that received an intravenous dose of 10 mg/kg/day (approximately 8 times the MRHD on a mg/kg basis). KALBITOR was not teratogenic in rats at subcutaneous doses up to 20 mg/kg/day (approximately 2.4 times the MRHD on an AUC basis) and rabbits that received intravenous doses up to 5 mg/kg/day (approximately 6 times the MRHD on an AUC basis).

### 14 CLINICAL STUDIES

The safety and efficacy of KALBITOR was evaluated in 2 randomized, double-blind, placebo-controlled trials (EDEMA4 and EDEMA3) in 168 patients with HAE. Patients having an attack of hereditary angioedema, at any anatomic location, with at least 1 moderate or severe symptom, were treated with 30 mg subcutaneous KALBITOR or placebo. Because patients could participate in both trials, a total of 143 unique patients participated. Of the 143 patients, 94 were female, 123 were Caucasian, and the mean age was 36 years. There were 64 patients with abdominal attacks, 55 with peripheral attacks, and 24 with laryngeal attacks.

In both trials, the effects of KALBITOR were evaluated using the Mean Symptom Complex Severity (MSCS) score and the Treatment Outcome Score (TOS). These measures evaluated the severity of attack symptoms at all anatomical locations (MSCS score) and response to therapy (TOS).

MSCS score is a point-in-time measure of symptom severity. At baseline, 4 hours, and 24 hours, patients rated the severity on a categorical scale (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for symptoms at each affected anatomical location. Ratings were averaged to obtain the MSCS score. A decrease in MSCS score reflected an improvement in symptoms.

TOS is a measure of symptom response to treatment. At 4 hours and 24 hours, patient assessment of response characterized by their change from baseline in symptom severity and collected by anatomic site of attack involvement, was recorded on a categorical scale (significant improvement [100], improvement [50], same [0], worsening [-50], significant worsening [-100]). The response at each anatomic site was weighted by baseline severity and then the weighted scores across all involved sites were averaged to calculate the TOS. A TOS value >0 reflected an improvement in symptoms from baseline.

#### **EDEMA4**

EDEMA4 was a randomized, double-blind, placebo-controlled trial in which 96 patients were randomized 1:1 to receive KALBITOR 30 mg subcutaneous or placebo for acute attacks of HAE. The primary endpoint was the change from baseline in MSCS score at 4 hours, and the TOS at 4 hours was a key secondary endpoint. Patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients with placebo and the results were statistically significant (Table 2). At 24 hours, patients treated with KALBITOR also demonstrated a greater decrease from baseline in the MSCS than placebo (-1.5 vs. -1.1; p = 0.04) and a greater TOS (89 vs. 55, p = 0.03).

Table 2: Change in MSCS Score and TOS at 4 Hours

•	EDEMA4		EDEMA3	
	KALBITOR	Placebo	KALBITOR	Placebo
	(N=48)	(N=48)	(N=36)	(N=36)
Change in MS	CS Score at 4 Hours			
n	47	42	34	35
Mean	-0.8	-0.4	-1.1	-0.6
95% CI	-1.0, -0.6	-0.6, -0.1	-1.4, -0.8,	-0.8, -0.4
P-value	0.010		0.041	
OS at.4 Hour	<u>'S</u>			
B	47	42	<b>34</b> .	35
Mean	53	8	63	36
95% CI	39, 68	-12, 28	49, 76	17, 54
P-value	0.003		0.045	

MSCS: Mean Symptom Complex Severity

**TOS: Treatment Outcome Score** 

CI: confidence interval

More patients in the placebo group (24/48, 50%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (16/48, 33%).

Some patients reported improvement following a second 30 mg subcutaneous dose of KALBITOR, administered within 24 hours following the initial dose for symptom persistence or relapse, but efficacy was not systematically assessed for the second dose.

#### **EDEMA3**

EDEMA3 was a randomized, double-blind, placebo-controlled trial in which 72 patients were randomized 1:1 to receive KALBITOR or placebo for acute attacks of HAE. EDEMA3 was similar in design to EDEMA4 with the exception of the order of the prespecified efficacy endpoints. In EDEMA3, the primary endpoint was the TOS at 4 hours, and the key secondary efficacy endpoint was the change from baseline in MSCS at 4 hours. As in EDEMA4, patients treated with KALBITOR demonstrated a greater decrease from baseline in the MSCS than placebo and a greater TOS than patients treated with placebo and the results were statistically significant (Table 2).

In addition, more patients in the placebo group (13/36, 36%) required medical intervention to treat unresolved symptoms within 24 hours compared to the KALBITOR-treated group (5/36, 14%).

#### 16 HOW SUPPLIED/STORAGE AND HANDLING

KALBITOR (ecallantide) is supplied as three 10 mg/mL single-use vials packaged in a carton. Each vial contains 10 mg of ecallantide. Each vial contains a slight overfill.

NDC (47783-101-01): 3 single-use vials in 1 carton

KALBITOR should be kept refrigerated (2°C to 8°C/36°F to 46°F). Vials removed from refrigeration should be stored below 86°F/30°C and used within 14 days or returned to refrigeration until use.

Protect vials from light until use.

Do not use beyond the expiration date.

Draft November 27, 2009

#### 17 PATIENT COUNSELING INFORMATION

- Patients should be advised that KALBITOR may cause anaphylaxis and other
  hypersensitivity reactions. Patients should be advised that KALBITOR should be
  administered by a healthcare professional with appropriate medical support to
  manage anaphylaxis and hereditary angioedema. Patients who have known
  clinical hypersensitivity to KALBITOR should be instructed not to receive
  additional doses of KALBITOR. [see Boxed Warning, Contraindications (4), and
  Warnings and Precautions (5.1)]
- Patients should be advised to consult the Medication Guide for additional information regarding the risk of anaphylaxis and other hypersensitivity reactions.

Confidential Page 10 of 10 Dyax Corp.

### Medication Guide

### KALBITOR® (KAL-bi-tor)

#### (ecallantide)

Read this Medication Guide before you start receiving KALBITOR and before each treatment. There may be new information. This Medication Guide does not take the place of talking to your doctor about your medical condition or your treatment.

### What is the most important information that I should know about KALBITOR? Serious allergic reactions may happen in some people who receive KALBITOR. These allergic reactions can be life-threatening and usually happen within 1 hour after receiving

KALBITOR.

· KALBITOR should be given to you by a doctor or nurse in a healthcare setting where serious allergic reactions and hereditary angioedema (HAE) can be treated.

- Symptoms of a serious allergic reaction to KALBITOR can be similar to the symptoms of HAE, the condition that you are being treated for. Your doctor or nurse should watch you for any signs of a serious allergic reaction after treatment with KALBITOR.
- Tell your doctor or nurse right away if you have any of these symptoms of a serious allergic reaction during or after treatment with KALBITOR:
  - wheezing, shortness of breath, cough, chest tightness, or trouble breathing
  - dizziness, fainting, fast or weak heartbeat, or feeling nervous
  - reddening of the face, itching, hives, or feeling warm
  - swelling of the throat or tongue, throat tightness, hoarse voice, or trouble swallowing
  - runny nose or sneezing

#### What is KALBITOR?

KALBITOR is a prescription medicine used to treat sudden attacks of hereditary angioedema (HAE).

KALBITOR is not a cure for HAE.

It is not known if KALBITOR is safe and effective in children under 16 years of age.

### Who should not receive KALBITOR?

Do not receive KALBITOR if you are allergic to KALBITOR.

# What should I tell my doctor before I receive KALBITOR?

Before receiving KALBITOR, tell your doctor if you:

- have ever had an allergic reaction to KALBITOR. See "Who should not take KALBITOR?"
- are pregnant or plan to become pregnant. It is not known if KALBITOR will harm your unborn baby.
- are breast-feeding or plan to breast-feed. It is not known if KALBITOR passes into your breast milk.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

Know the medicines you take. Keep a list of them to show to your doctor and pharmacist when you get a new medicine.

### How will I receive KALBITOR?

For each dose, you will receive 3 injections just under the skin (subcutaneous or SC injections) of your abdomen, thigh, or upper arm.

### What are the possible side effects?

KALBITOR can cause serious allergic reactions. See "What is the most important information I should know about KALBITOR?").

Common side effects of KALBITOR include:

- headache
- nausea
- diarrhea
- fever
- · injection site reactions, such as redness, rash, swelling, itching, or bruising
- stuffy nose

Call your doctor for advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

#### General information about KALBITOR

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide. This Medication Guide gives you the most important information about KALBITOR. If you would like more information, talk with your doctor. You can ask your pharmacist or doctor for information about KALBITOR that is written for health professionals.

### What are the ingredients of KALBITOR?

Active Ingredient: ecallantide

Inactive ingredients: disodium hydrogen orthophosphate (dihydrate), monopotassium phosphate, potassium chloride, sodium chloride in water for injection.

Manufactured for: Dyax Corp.

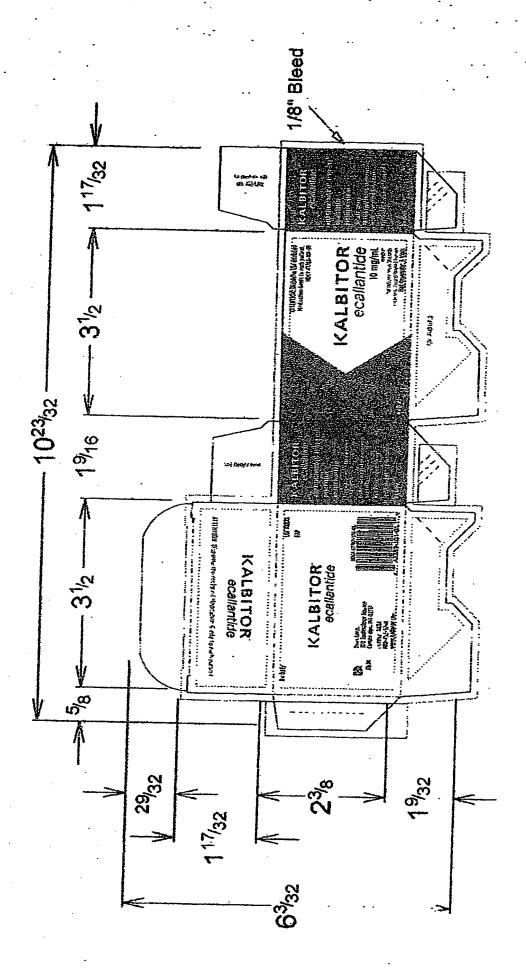
300 Technology Square, Cambridge, MA 02139

Issued Month Year

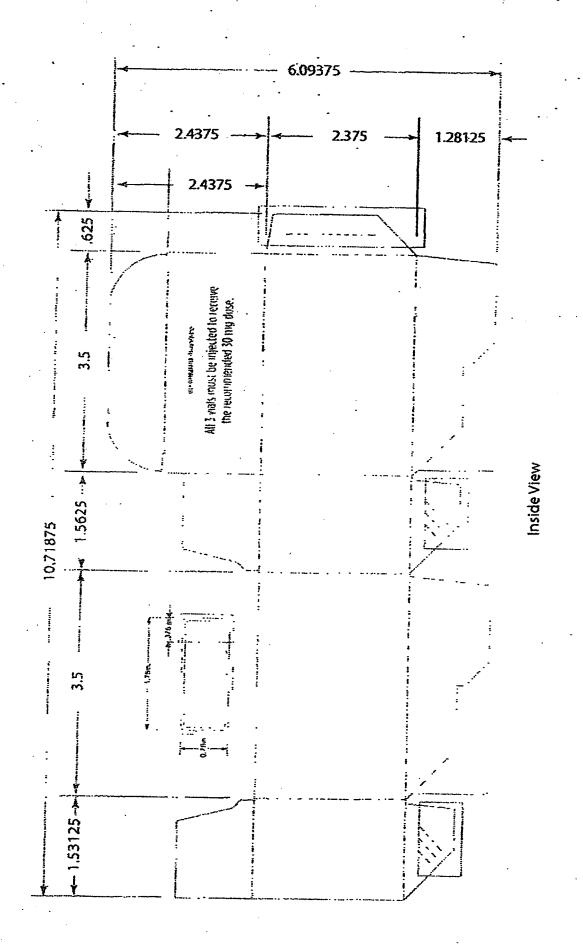
This Medication Guide has been approved by the U.S. Food and Drug Administration.

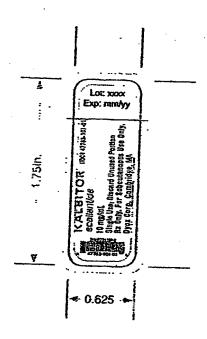
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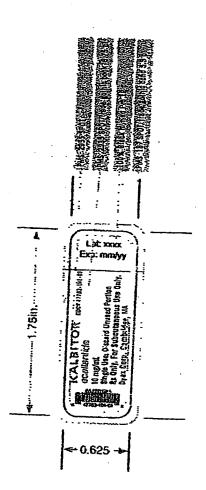
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יינה זיתן אותי לאונית כאון למש הן נו







Lot: xxxx Exp: mm/yy

NDC# 47783-101-01

ecallantide

10 mg/mL Single Use; Discard Unused Portion Rx Only. For Subcutaneous Use Only. Dyax Corp. Cambridge, MA

0.625

In re U.S. Patent No.: 7,276,480 \_\_.

Attorney Docket No.: D2033-7. 12 US/10280

094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment E

Terminal Disclaimer

Attorney's Docket No.: 10280-( 03 / HB-02-04 DIV

#### ATTACHMENT E

#### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Robert C. Ladner et al.

Art Unit : 1656

Serial No.: 11/323,261

Examiner: Marsha M. Tsay

Filed

: December 30, 2005

Conf. No.: 5442

Title

: PREVENTION AND REDUCTION OF BLOOD LOSS

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

#### TERMINAL DISCLAIMER UNDER 37 C.F.R. §§ 3.73(b) AND 1.321(b)

Pursuant to 37 C.F.R. § 3.73(b), DYAX CORP., a corporation, certifies that it is the assignce of the entire right, title, and interest in the above-referenced application by virtue of:

X An assignment from the inventors of the above-referenced patent application. The assignment was recorded in the Patent and Trademark Office at Reel 017499, Frame 0493 on April 18, 2006, or a copy thereof is attached.

To the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

Pursuant to 37 C.F.R. § 1.321(b), and to obviate a double patenting rejection, the assignee identified above hereby waives and disclaims the terminal portion of the term of the entire patent to be granted upon the above-referenced application subsequent to the expiration date of the patent to issue from U.S. Patent Application Serial No.: 10/456,981 provided that any patent granted on the above-referenced application shall be enforceable only for and during such period that it is commonly owned with the patent to issue from U.S. Patent Application Serial No.: 10/456,981.

Applicant: Robert C. Ladner et al.

Serial No.: 11/323,261

Filed:

: December 30, 2005

Page

: 2 of 2

The assignce identified above does not disclaim any terminal part of any patent granted on the above-referenced application prior to the expiration date of the full statutory term of the patent to issue from U.S. Patent Application Serial No.: 10/456,981 in the event that it later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. § 1.321(a), has all claims cancelled by a reexamination certificate, or is otherwise terminated prior to expiration of its statutory term, except for the separation of legal title as stated above. Assignee herein does not disclaim or otherwise affect any part of the patent to issue from U.S. Patent:Application Serial No.: 10/456,981

This disclaimer runs with any patent granted on the above application and is binding upon the grantee, its successors or assigns.

Enclosed is a check for \$130 for the required fee pursuant to 37 C.F.R. § 1,20(d). Please apply any other charges or credits to Deposit Account No. 06-1050.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: JAN. 15, 2002

Ivana Magovcevic-Liebitsch

Title:

DYAX CORP.

Fish & Richardson P.C. 225 Franklin Street Boston, MA 02110

Telephone: (617) 542-5070 Facsimile: (617) 542-8906

21531642.doc

Ivana Magovčević-Liebisch, Ph.D., J.D. General Couńsel & Executive Vice President, Corporate Communications

Attorney's Docket No.: 10280-094003 / HB-02-04 DIV

In re U.S. Patent No.: 7,276,486 Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attorney Docket No.: D2033-: \$\frac{12}{912}\$ US/10280-094003

Attachment F

Certificate of Correction

#### ATTACHMENT F

#### UNITED STATES PATENT AND TRADEMARK OFFICE



DEC 3 1 2007

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

HELLER EHRMAN LLP 275 MIDDLEFIELD ROAD MENLO PARK CA 94025-3506

In re Patent No. 7,276,480 LADNER et al

Issue Date: October 2, 2007

Appl No.: 11/323,261 Filed: December 30, 2005

For: Correction of Inventorship

**DECISION GRANTING** 

PETITION 37 CFR 1.324

This is a decision on the petition filed October 12, 2007, to correct inventorship under 37 CFR 1.324.

The petition is **GRANTED**.

The patented file is being forwarded to Certificate of Corrections Branch for issuance of a certificate naming only the actual inventor or inventors.

William R. Dixon, Jr.

Special Program Examiner Technology Center 1600



#### UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

DATE:

December 27, 2007

TO:

Certificates of Correction Branch

FROM:

William R. Dixon, Jr.

**Technology Center 1600** 

SUBJECT:

REQUEST FOR CERTIFICATE OF CORRECTION

Please issue a Certificate of Correction in U. S. Letters Patent No. 7,276,480 as specified on the attached Certificate.

Please put Julie Burke as the signatory official on the certificate as I will be retired by the time the certificate is prepared.

William R. Dixon, Jr.

**Technology Center 1600** 

#### UNITED STATES PATENT AND TRADEMARK OFFICE

#### **CERTIFICATE**

Patent No. 7,276,480 Patented: October 2, 2007

On petition requesting issuance of a certificate for correction of inventorship pursuant to 35 U.S.C. 256, it has been found that the above identified patent, through error and without deceptive intent, improperly sets forth the inventorship. Accordingly, it is hereby certified that the correct inventorship of this patent is:

Robert C. Ladner Arthur C. Ley

William R. Dixon, Jr.

Special Program Examiner Technology Center 1600 In re U.S. Patent No.: 7,276,480

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attorney Docket No.: D2033-7 12 US/10280-

094003

Attachment G

Maintenance Fees Status









#### ATTACHMENT G



Patent Maint	enance Fees	12/29/2	009 01:46 PM EST
Patent Number:	7276480	Application Number:	
Issue Date:	10/02/2007	Filing Date:	12/30/2005
Window Opens:	10/04/2010	Surcharge Date:	04/05/2011
Window Closes:	10/03/2011	Payment Year:	
Entity Status:	LARGE		
<b>Customer Number</b>	37462		
Street Address:	LANDO & ANASTA	ASI, LLP	
City:	CAMBRIDGE		
State:	MA		
Zip Code:	02142	· · · · · · · · · · · · · · · · · · ·	
Phone Number:	(617) 395-7000		
	Currently th	ere are no fees du	ıe.

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Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attorney Docket No.: D2033-( 094003

Attachment H1

Letter from FDA acknowledging receipt of the first IND



wellved 30 Jan 2002

Food and Drug Administration 1401 Rockville Pike Rockville MD 20852-1448

JAN 1 8 2002

Our Reference: BB-IND 10232

Dyax Corporation
Attention: Lynn G. Baird, Ph.D.
Senior Vice President, Preclinical and Regulatory Affairs
300 Technology Square
Cambridge, MA 02139

Dear Dr. Baird:

The Center for Biologics Evaluation and Research has received your Investigational New Drug Application (IND). The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 10232

SPONSOR: Dyax Corporation

PRODUCT NAME: Plasma Kallikrein Inhibitor (recombinant, *Pichia pastoris*, Avecia Biotechnology)

DATE OF SUBMISSION: January 10, 2002

DATE OF RECEIPT: January 11, 2002

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an original and two copies of every submission to this file. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-4358. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research Attn: Office of Therapeutics Research and Review HFM-99, Room 200N 1401 Rockville Pike Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Karen D. Winestock

Regulatory Project Manager

Division of Application Review and Policy

Office of Therapeutics

Research and Review

Center for Biologics

.Evaluation and Research

Enclosures (3): 21 CFR Part 312

21 CFR 50.20, 50.25

Information sheet on 21 CFR 25.24

In re U.S. Patent No.: 7,276,486 \_1

Attorney Docket No.: D2033-7. 312 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment H2

Contact Report for DYAX-FDA Teleconference of February 8, 2002

### ATTH. LHMENT HZ

#### **RECORD OF CONTACT**

**CONTACT:** Scott Proestel

DATE OF CONTACT: 08 February 2002 DATE OF REPORT: 08 February 2002

DYAX: Lynn G. Baird, Tony Williams,

IND#: 10232

Jeff Peart

cc: Henry Blair, Shil Hirani, Tony Williams, Jeff Peart

**SUBJECT:** Stopping Rule

We called Scott Proestel on 08 February 2002. He acknowledged that he had received our fax. He said that our proposed stopping rule looked satisfactory to him and he was scheduled to review it with his supervisor in the next half hour or so. He would get back to us after they had conferred.

In the fax, we indicated that we would like to clarify the outcome of yesterday's discussion on replacement of patients. Scott said that all patients who are treated on study should be followed for 28 days for safety. Patients who do not receive study medication can be replaced; those who have receive study medication should not be replaced. Tony asked if we could replace a patient who was treated but from whom no PK samples were collected. Scott did not think such patients should be replaced, although he offered to discuss the matter with his supervisor if we wanted. Lynn suggested that rather than trying to define appropriate exceptions in the protocol, we consider calling Scott to discuss any patient for whom we thought replacement was justified. Tony agreed to replace only non-dosed patients and to call if he felt there were justifiable exceptions.

Scott said that he would call in a half an hour or so about the stopping rule after talking with Ellis Unger. He called and said everything looked satisfactory with the exception of one number. The proposed SAE trigger of greater than 3 of 4 was too high. They recommended setting this trigger at 2 of 4. I agreed that we would make the change. Scott said that we could proceed with our Phase I/II clinical trial. He asked that we submit the amended protocol as an IND amendment. I assured him that we would.

In re U.S. Patent No.: 7,276,480

Attorney Docket No.: D2033-094003

112 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment I

Letter from FDA acknowledging receipt of the second IND

#### **ACHMENT I**



### DEPARTMENT OF HEALTH & HUMAN SERVICES

MAY 1 0 2002

Food and Drug Administration 1401 Rockville Pike Rockville MD 20852-1448

Our Reference: BB-IND 10426

Dÿax Corporation Attention: Lynn G. Baird, Ph.D. Senior Vice President, Development 300 Technology Square Cambridge, MA 02139

Dear Dr. Baird:

The Center for Biologics Evaluation and Research has received your Investigational New Drug Application (IND). The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND#: 10426

SPONSOR: Dyax Corporation

PRODUCT NAME: Kallikrein Plasma Inhibitor (recombinant, Pichia pastoris, Avecia

Biotechnology)

DATE OF SUBMISSION: April 30, 2002

DATE OF RECEIPT: May 1, 2002

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an original and two copies of every submission to this file. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent

regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-4358 Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research Attn: Office of Therapeutics Research and Review HFM-99, Room 200N 1401 Rockville Pike Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Pames Reese for Karen Winestock

Regulatory Project Manager

Division of Application Review and Policy

Office of Therapeutics

Research and Review

Center for Biologics

**Evaluation and Research** 

Enclosures (3): 21 CFR Part 312

21 CFR 50.20, 50.25

Information sheet on 21 CFR 25.24

In re U.S. Patent No.: 7,276,480

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

#### Attachment J

Letter from Dyax to the Center for Biologic Evaluation and Research dated May 31, 2002 which summarized the May 30, 2002 telephone conference

Attorney Docket No.: D2033-7

094003

# AT ACHMENT J

	DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION	Form Approved: OMB I Expiration Date: Septem See OMB Statement on	ber 30, 2002
	INVESTIGATIONAL NEW DRUG APPLICATION (IND) (IIILE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)	NOTE: No drug may investigation begun tovestigation is in effect	be shipped or clinical until an IND for that (21 CFR 312.40).
١	1. NAME OF SPONSOR	2. DATE OF SUBMISSION	
٠	Dyax Corp.	31 May 2002	
1	3. ADDRESS (Number, Street, City, State and Zip Code)	4. TELEPHONE NUMBER	•
	300 Technology Square Cambridge, MA 02139	(Include Area Code) 617-250-5705	
	6. NAME(S) OF DRUG (Include all available names: Trade, Generic, Chemical, Code)	6. IND NUMBER (If previous BB-IND # 10426	usly assigned)
ŀ	DX-88 (Recombinant Human Plasma Kallikrein Inhibitor)  7. INDICATION(S) (Covered by this submission)		······································
ı	1. INDICATION(S) (Covered by this submission)		· · · .
1	Hereditary Angioedema	•	•
ŀ	8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED:		· · · · · · · · · · · · · · · · · · ·
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1	B. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS (21 CFR Part 3	12), NEW DRUG OR A	NTIBIOTIC APPLICATIONS
	(21 CFR Part 314), DRUG MASTER FILES (21 CFR Part 314,420), AND PRODUCT LICENS TO INTHIS APPLICATION.	SE APPLICATIONS (21 C	FR Part 601) REFERRED
١	BB-IND # 10232		
		•	
	10. IND submission should be consecutively numbered. The initial IND sh	ould be numbered	
	"Serial number: 000." The next submission (e.g., amendment, report, of should be numbered "Serial Number: 001." Subsequent submission	r correspondence)	SERIAL NUMBER
7	numbered consecutively in the order in which they are submitted.	ad ninolie sliok	0 0 2
-	. :  11. THIS SUBMISSION CONTAINS THE FOLLOWING: (Check all that apply)		
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	☐ NEW PROTOCOL ☐ CHEMISTRY/MICROBIOLOGY	INITIAL WRITTEN RE	PORT
	☐ CHANGE IN PROTOCOL ☐ PHARMACOLOGY/TOXICOLOGY	FOLLOW-UP TO A W	RITTEN REPORT
1'	NEW INVESTIGATOR CLINICAL		
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1 16.	F APPLICATION owing items: (Check all that apply)
1. Form FDA 1571 [21 CFR 312.23(a)(1)]	
2. Table of Contents [21 CFR 312.23(a)(2)]	•
3. Introductory statement [21 CFR 312.23(a)(3)]	
_	•
4. General Investigational plan [21 CFR 312.23(a)(3)]	
5. Investigator's brochure [21 CFR 312.23(a)(5)]	
☐ 6. Protocol(s) [21 CFR 312.23(a)(6)]	
a. Study protocol(s) [21 CFR 312.23(a)(6)]	
b. Investigator data [21 CFR 312.23(a)(6)(iii)(b)]	
☐ c. Facilities data [21 CFR 312.23(a)(6)(iii)(b)] or	
d. Institutional Review Board data [21 CFR 312	
7. Chemistry, manufacturing, and control data [21 CFR 312.2]	·
Environmental assessment or claim for exclusio	n [21 CFR 312.23(a)(7)(iv)(e)]
8. Pharmacology and toxicology data [21 CFR 312.23(a)(8)]	•
9. Previous human experience [21 CFR 312.23(a)(9)]	•
10. Additional information [21 CFR 312.23(a)(10)]	
13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRU	•
IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CO	·
IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS O IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIG	IF THE CONTRACT RESEARCH ORGANIZATION, ATIONS TRANSFERRED.
NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE	CONDUCT AND PROGRESS OF THE CLINICAL
Anthony H. Williams, MA, MRCP, MB, MS	
Sr. Vice President	
Medical Affairs and Clinical Operations	
15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW A	NID EVALUATION OF INFORMATION RELEVANT TO THE
SAFETY OF THE DRUG	
Anthony H. Williams, MA, MRCP, MB, MS Sr. Vice President	Lynn G. Baird, PhD Sr. Vice President
Medical Affairs and Clinical Operations	Development
lagree not to begin clinical investigations until 30 days after	r FDA's receipt of the IND unless I receive earlier notification gin or continue clinical investigations covered by the IND if
	in Institutional Review Board (IRB) that compiles with the
requirements set fourth in 21 CFR Part 56 will be responsible	e for initial and continuing review and approval of each of the
	duct the investigation in accordance with all other applicable
regulatory requirements.  16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED	17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED
REPRESENTATIVE	REPRESENTATIVE
Lynn G. Baird, PhD	
Sr. Vice President	JKM D Bard
Development	10 TH COUNTY AND DOOR
18. ADDRESS (Number, Street, City, State and Zip Code)  Dyax Corp.	19. TELEFHONE NUMBER 20. DATE (Include Area Code)
300 Technology Square	617-250-5705
Cambridge, MÃ 02139	617-250-5705 31 May 2002
QVARNING: A willfully false statement is a criminal offense. U.S.C. Title 18, Sec	1 U U U U U U U U U U U U U U U U U U U
this reporting hurrier for this collection of information is estimated to ave	erage 100 hours per response, including the time for reviewing instructions,
Seamhing existing data sources, gathering and maintaining the data needs	ed, and completing reviewing the collection of Information. Send comments
regarding this burden estimate or any other aspect of this collection of information	

CBER (HFM-99) 1401 Rockville Pike Rockville, MD 20852-1448

CDER (HFD-94) 5516 Nicholson Lane Kensington, MD 20895

person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

Dyax Corp.



31 May 2002

**BB-IND # 10426, Serial 002** 

Dr. Jay Siegel
Center for Biologics Evaluation and Review
HFM-99, Room 200N
Attention: Office of Therapeutics Research and Review
1401 Rockville Pike
Rockville, MD 20852-1448

Re: DX-88 (Recombinant Human Plasma Kallikrein Inhibitor) for Treatment of

Hereditary Angioedema

Attention: Karen Winestock

Dear Dr. Siegel:

This letter is a summary of a teleconference on 30 May 2002 with Anita O'Connor and Scott Proestel, and a follow-up of a teleconference on 29 May 2002 (reference BB-IND # 10426, Serial 001). Teleconference participants representing Dyax were Lynn Baird, SVP Development, Tony Williams, SVP Medical Affairs and Clinical Operations, and Jeff Peart, Manager Regulatory Affairs.

Dr. Proestel and Ms. O'Connor had reviewed a facsimile copy of Serial 001 and had four follow-up comments/questions:

1. Regarding the requested change from an absolute milligram dose in the proposed study to a dose based on mg/ m² body surface, what numerical doses are being considered?

Dr. Williams indicated that doses from of 5, 10, 20, 40 mg/ m² body surface would be used. He had selected these doses based on the originally proposed doses of 10, 20, 40, and 80 mg and a surface area of an average person of 1.92 m². An upper limit for surface area of 2.5 m² will be used to prevent potential overdosing.

2. It is suggested that the stopping rule associated with patient death apply to all deaths rather than "medical" deaths. Alternatively, specific events leading to

death could be identified. This should not delay study enrollment as CBER review of unrelated deaths will be rapid.

Dyax agrees to write the stopping rule to include all deaths.

3. Dr. Proestel clarified that it would be acceptable to treat up to fifty patients, not episodes, in the proposed study.

Dyax agrees. However, the protocol as written allows for treatment of 48 attacks, and will be amended to treat 48 patients.

4. Dr. Proestel asked for clarification regarding how we intended to deal with potential retreatments.

Dr. Williams stated that patients would be treated under a separate protocol rather than under the existing protocol for administrative reasons.

Dr. Proestel confirmed that the clinical trial could be initiated. However, Dyax must submit a revised clinical protocol to the IND before patient enrollment begins. Dyax agreed to submit the protocol in a subsequent amendment.

Please contact me by telephone at 617-250-5705 or telefax at 617-225-2501 if you need any additional information or require any clarification.

Sincerely.

Lynh/G. Baird, Ph.D. Senior Vice President

Development

In re U.S. Patent No.: 7,276,480

Attorney Docket No.: D2033-7 12 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

#### Attachment K

Communication dated June 12, 2008 from Dyax Corp. to the Center for Drug Evaluation and Research discussing conveyance of BB-IND#10232 to Cubist **Pharmaceuticals** 

### ATTACHMENT K

DEPARTMEN	NT OF HEALTH AND HUMAN SERVICES	En America		
	OU AND DRUG ADMINISTRATION	Form Approved: OMB No. 0910-0430. Expiration Date: April 30, 2009 See OMB Statement on Reverse.		
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Dyax Corp.		2 DATE OF SUBMISSION		
3. ADDRESS (Number, Street, City, S	State and Zip Code)	06/12/2008		
300 Technology Square Cambridge, MA 02139	•	4. TELEPHONE NUMBER (Include Area Code)		
5. NAME(SLOF DRING (Institute of		617-250-5773		
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7. INDICATION(S) (Covered by this su	function	10232		
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1	1. Form FDA 1571 [21 CFR 31.	2.23(a)(1)]	•		•
	2. Table of Contents [21 CFR 3	12.23(a)(2)]			•
-	L. 3. Introductory statement [21 C	FR 312.23/aV311		•	
•	4. General Investigational plan	21 CFR 312 23/21/211		•	•
•	5. Investigator's brochure [21 C	ED 210 000 vers			
	☐ 6. Protocol(s) [21 CFR 312.23(a	m sizza(ajįs)j	1	•	• • •
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- 1	Li a. Study protocost	s) [21 CFR 312.23(a)(6)]	• •	• • •	•
•	Li b. Investigator data	21 CFR 312.23(a)(6)(iii	7)(b)] or completed Form	in COLETA ACTO	
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-1				7 FUA 15/2	•
- 11	7. Chemistry, manufacturing, and	iew Board data [21 CFR at control data [21 CFR at	<i>いこと</i> (a)(o)(即)(b)] Or O	ompleted Form(s)	FDA 1572
· }	· D Environmental ass	essent or object of the	223(a)(7)]		•
	8. Pharmacology and toxicology	essment or claim for excl	usion [21 CFR 312.23(a	i)(7)(iv)(e)].	
- 10	7 9. Previous hymnin american co	ыа (21 CFH 312.23(a)(6	3)]		•
10	9. Previous human experience (2	1 CFH 312.23(a)(9)]			-
15	10. Additional information [21 CFR	312.23(a)(10)]	•		
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300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



12 June 2008

IND # 10232, Serial 100

Dwayne Rieves, MD
Therapeutic Biological Products Document Room
Center for Drug Evaluation and Research
Food and Drug Administration
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: Ecallantide IV: In Patients Undergoing Cardiopulmonary Bypass Procedures Ecallantide (DX-88 [Recombinant Human Plasma Kallikrein Inhibitor])
General Correspondence: Notice of Transfer of IND # 10232

Dear Dr. Rieves:

The purpose of this submission is to notify the FDA that sponsorship of IND 10232, DX-88 (ecallantide) for the reduction of blood loss for patients undergoing cardiopulmonary bypass surgery, is being transferred to Cubist Pharmaceuticals, Inc as of June 16, 2008. All documents associated with IND 10232 have been transferred to Cubist and all active investigators will be notified that Cubist is assuming the sponsor responsibilities for ecallantide as of June 16, 2008.

On June 16, Cubist will also assume responsibility for the ongoing clinical study DX-88/16, entitled "KALAHARI 1: Kallikrein Antagonist (DX-88 [Ecallantide]) Effect on Blood Loss Associated with Heart Surgery Requiring Institution of Bypass".

In a separate submission to IND 10232, Cubist Pharmaceuticals, Inc. will notify the FDA that they assume all of the sponsor responsibilities for IND 10232 as of June 16, 2008. Included in the Cubist submission will be a completed FDA 1571 Form which indicates the contact information for Cubist.

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or Aurelie Grienenberger by telephone at 617-250-5762 or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely

Nicole D'Auteuil \ Senior Director of Regulatory Affairs

Submitted in triplicate

Confidential

Page 1 of 1

In re U.S. Patent No.: 7,276,480

Attorney Docket No.: D2033-7-312 US/10280-094003

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

#### Attachment L

Communication from Dyax Corp. to the Center for Drug Evaluation and Research dated June 13, 2008, in which BB-IND#10426 was amended

### ATT CHMENT L

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7. IMUICATION(S) (Covered by this submission)	· · · · · · · · · · · · · · · · · · ·	10426			
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12. CONTENTS	OF APPLICATION
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1. Form FDA 1571 [21 CFR 312.23(a)(1)]	
2. Table of Contents [21 CFR 312.23(a)(2)]	
3. introductory statement [21 CFR 312.23(a)(3)]	
4. General Investigational plan [21 CFR 312.23(a)(3)]	
5. Investigator's brochure [21 CFR 312.23(a)(5)]	
6. Protocol(s) [21 CFR 31223(a)(6)]	
a. Study protocol(s) [21 CFR 312.23(a)(6)]	
☐ b. Investigator data [21 CFR 312.23(a)(6)(iii)(b	at an armediate of Plantical Place of Plantical Plantica
口 c. Facilities data [21 CFR 31223(a)(6)(ii)(b)](	// Or completed norms/ num 1972
d. Institutional Review Board data [21 CFR 31]	OF COMPRESENTING STOP 10/2
7. Chemistry, manufacturing, and control data [21 CFR 312]	2.23(a)(6)(w)(b)) or completed Form(s) FDA 15/2
Environmental assessment or claim for exclusi	23(a)(7))
8. Pharmacology and toxicology data [21 CFR 312-23(a)(8)]	on[27 G-H 31223(a)(/)(N)(0)]
9. Previous human experience [21 CFR 312.23(a)(9)]	
10. Additional information [21 CFR 312.23(a)(10)]	•
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14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE INVESTIGATIONS	
Patrick Horn, M.D., Ph.D.	
Sr. Medical Director, Dyax Corp.	
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15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW / SAFETY OF THE DRUG	AND EVALUATION OF INFORMATION RELEVANT TO THE
Bill Pullman, M.D., Ph.D.	
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requirements.	estigation in accordance with all other applicable regulatory
6. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE	17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE
Nicole D'Auteuil	REPRESENTATIVE
Senior Director of Regulatory Affairs	WORDANK
B. ADDRESS (Number, Street, City, State and Zip Code)	19. TELEPHONE NUMBER 20. DATE
Dyax Corp.	(Include Area Code) 05/13/2008
300 Technology Square Cambridge, MA 02139	617-250-5773
VARMING: Awaitully false statement is a criminal offense. U.S.C. Title 18, Sec.	1001)
utilic reporting burden for this collection of information is estimated to average 100	house not recovered including the time for multiplication included in the second
ther aspect of this collection of information, including suggestions for reducing this bu	the conection of information. Send comments regarding this burden estimate or any inden to:
epartment of Health and Human Services Department of Health and Human Services Pool and Drug Administration Food and Drug Administration	man Services
Food and Drug Administration Food and Drug Administration Center for Biologics Evaluation	"An agency may not conduct or sponsor, and a n and Research (HFM-99) person is not required to respond to, a collection
entral Document Room 1401 Rockville Pike 01-8 Ammendale Road Rockville, MD 20852-1448	of information unless it displays a currently valid OMB control number."
fisville, MD 207052-1266	application to this address.

300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



13 June 2008

IND # 10426, Serial 193

Badrul Chowdhury, M.D., Ph.D. Division of Pulmonary and Allergy Products Center for Drug Evaluation and Research Food and Drug Administration Therapeutic Biological Products Document Room 5901-B Ammendale Road Beltsville, MD 20705-1266

DX-88 (ecallantide) for Treatment of Angioedema

Re-submission of documentation

Letter of Authorization to Cross-Reference IND 10426

Dear Dr. Chowdhury:

We are re-submitting information that was previously submitted to the Agency. The current submission is being made due to an IND transfer to a new sponsor. Dyax Corp. has been the Sponsor of INDs for DX-88 (ecallantide) in two indications:

- IND 10426 for attacks of hereditary angioedema (Division of Pulmonary and Allergy Products); and
- IND 10232 for the reduction of blood loss for patients undergoing cardiopulmonary bypass surgery (Division of Medical Imaging and **Hematology Products**)

Dyax is retaining sponsorship of IND 10426, however, transferring sponsorship of IND 10232 to Cubist Pharmaceuticals, effective June 16, 2008. Due to the transfer of IND 10232, Dyax is re-submitting all Quality and Nonclinical (NC) documentation that was previously submitted to IND 10232 and cross-referenced to IND 10426. The information in the current submission is entirely duplicative to prior submissions and is provided for the purpose of ensuring that documentation previously submitted to only IND 10232 is now directly incorporated to IND 10426.

Also attached is a letter of authorization to cross-reference IND 10426 on behalf of submissions made by Cubist Pharmaceuticals in association with IND 10232.

As agreed with the IND project manager, Akilah Green, only 1 copy of this submission is provided because there is no new information for review. The attached table of contents lists the documentation included in the current resubmission.

Future Quality and NC submissions in support of the HAE program will be fully submitted to IND 10426.

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or Aurelie Grienenberger at 617-250-5762 or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely,

Nicole D'Auteuil

Senior Director of Regulatory Affairs

Submitted in triplicate

#### Table of Contents IND 10426, Serial 193 Re-Submitted Documentation & IND Letter of Authorization

IND 10426 S-193 Volume #	IND 10232 Submission date	IND 10232 Serial#	CMC/NC	Brief description
1		Not applicable		1571 Cover letter IND letter C. II
1-3	10 Jan 2002	000	CMC/NC	Sections 7 and 8 of original IND (Volume 1, 3 and 4-Att8 only)
4	30 Jan 2002	001	NC	Clarification to animal identification numbers
5	01 Feb 2002	002	CMC	Animal origin statement
6	06 Feb 2002	003	CMC	HPLC and SDS-PAGE data of DP lots used in toxicology and clinic
7	12 Aug 2002	005	CMC	Comparability of manufacturing scale-up from 100L to 1000L
8	13 Nov 2002	011	CMC	Response to FDA request for additional DS an
9	30 Dec 2002	013	NC	DP testing
10	07 May 2003	018	CMC	Nonclinical study report
11	24 Oct 2003	022	CMC	DS and DP shelf life extension  New RP-HPLC method
12	21 Apr 2004	024	NC	Leberter wind
13	26 Apr 2004	025	CMC	Laboratory animal adverse events  DX-88 purification process modification to
14-17	21 May 2004	027	NC	reduce host cell proteins
18	01 Jun 2004	028	NC	Nonclinical study reports
19	14 Jul 2004	031	NC	Nonclinical pathology review of rat deaths Synopsis of proposed NC study to support manufacturing change
20	21 Sept 2004	035	NC	I aborators animal al
21	08 Dec 2004	038	CMC/Antib ody	Laboratory animal adverse event  Response to FDA comments regarding DS and DP testing
22-23	14 Mar 2005	039	NC NC	
24	15 Apr 2005	041	NC NC	Nonclinical study report  Rationale for rat as relevant species for
25	26 Apr 2005	042	NC	Summary of nonclinical laboratory deaths as we as animal adverse event narratives
26	7 Jun 2005	043	CMC	Response to EDA managers
27	20 Jun 2005	044	CMC	Response to FDA request for quality informatio
28-29	30 Aug 2005	046	CMC/NC	Qualification of master seed bank (MSB)  Comparability data for 1000L and 5000L manufacturing processes, changes in drug product specifications
30	04 Oct 2005	047	CMC	DX-88 extinction coefficient and ultrafiltration step in manufacturing
31-42	12 Oct 2006	053	NC	Nonclinical study reports
43	1 Nov 2006	056	CMC	DP and DS changes to specifications and reference standard data
44	16 Nov 2006	057	CMC	Response to FDA request for information
45	27 Dec 2006	060	CMC	following EOP1/Pre-Phase 2 Meeting
16-49	9 Apr 2007	069	NC	Description of placebo Annual Report to 10232, including NC study reports
50	23 Apr 2007	070	CMC	Certificate of analysis of DS and DP lots in clinical studies

IND 10426 S-193 Volume #	IND 10232 Submission date	IND 10232 Serial #	CMC/NC	Brief description
	13 Feb 2008	089	CMC	Manufacturer comparability, reference standard
52-55	9 April 2008	094	NC	qualification, pH investigations Annual Report to 10232, including NC study reports

300 Technology Square Cambridge, MA 02139 phone (617) 225-2500 fax (617) 225-2501

Dyax Corp.



#### 13 June 2008

Badrul Chowdhury, M.D., Ph.D.
Division of Pulmonary and Allergy Products
Center for Drug Evaluation and Research
Food and Drug Administration
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: Letter of Authorization to Cross Reference IND 10426

Dear Dr. Chowdhury:

Dyax Corp. authorizes the FDA to incorporate, by reference, information in IND 10426 in consideration of INDs or other regulatory submissions filed by Cubist Pharmaceuticals. IND 10426 is current and up-to-date.

Reference:

IND 10426

Authorization on behalf of:

Cubist Pharmaceuticals, Inc.

65 Hayden Ave

Product:

Lexington, MA 02421 DX-88 (ecallantide)

Application type:

IND or other regulatory submissions for ecaliantide

Please contact me by telephone at 617-250-5773 or by email at <a href="mailto:ndauteuil@dyax.com">ndauteuil@dyax.com</a> or by email at <a href="mailto:agrienenberger@dyax.com">agrienenberger@dyax.com</a> if you need any additional information or require any clarification.

Sincerely.

Nicole D'Auteuil

Senior Director of Regulatory Affairs

In re U.S. Patent No.: 7,276,486 \_1

Attorney Docket No.: D2033- 812 US/10280-

Issued: October 2, 2007

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment M

Letter from FDA acknowledging receipt of the final submission of the BLA



## ATTACHMENT M DEPARTMENT OF HEALTH & HUMAN SERVICE

Public Health Service

Food and Drug Administration Rockville, MD 20857

Our STN: BL 125277/0

BLA ACKNOWLEDGEMENT

OCT 29 2008

Dyax Corporation 300 Technology Square Cambridge, MA 02139

Attention:

Nicole D'Auteuil

Senior Director, Regulatory Affairs

Dear Ms. D'Auteuil:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following:

Name of Biological Product: KALBITOR (ecallantide) Injection

Date of Application: September 23, 2008

Date of Receipt: September 23, 2008

Our Submission Tracking Number (STN): BL 125277/0

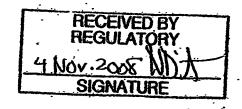
Proposed Use: Treatment of Hereditary Angioedema

If you have not already done so, promptly submit the content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format as described at <a href="http://www.fda.gov/oc/datacouncil/spl.html">http://www.fda.gov/oc/datacouncil/spl.html</a>. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action. The content of labeling must conform to the format and content requirements of revised 21 CFR 201.56-57.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration Center for Drug Evaluation and Research 5901-B Ammendale Road Beltsville, MD 20705-1266



All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unmecessary delay in processing which could have an adverse impact on the review of the submission.

If you have any questions, call me at (301) 796-1230.

Sincerely,

Colette Jackson

Regulatory Health Project Manager

Division of Pulmonary and Allergy Products

Office of Drug Evaluation II

Center for Drug Evaluation and Research

DEPARTMENT OF
HEALITH & HUMAN SERVICES
Food and Drug Administration
Center for Drug Evaluation Research,
Central Document Room
5901–B Ammendale Road
Beltsville, MD 20705–1266

Official Business Penalty for Private Use \$300



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In re U.S. Patent No.: 7,276,486 Susued: October 2, 2007

Attorney Docket No.: D2033- 312 US/10280-094003

Inventors: Robert C. Ladner and Arthur C. Ley

Assignee: Dyax Corp.

Title: PREVENTION AND REDUCTION OF BLOOD LOSS

Attachment N

Certification of Copies of Application Papers